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(54) Title: SYNTHESIS OF COMBINATORIAL ARRAYS OF ORGANIC COMPOUNDS THROUGH THE USE OF MULTIPLE COMPONENT COMBINATORIAL ARRAY SYNTHESES

(57) Abstract

The present invention relates to an array of compounds having a common core structure wherein the compounds of the array comprise the products of a multiple component combinatorial array synthesis having at least three components. The present invention also relates to the method of synthesizing that array. A further embodiment of the present invention relates to the use of solid phase synthesis to synthesize the combinatorial array of compounds. The present invention also relates to a method of creating a combinatorial array of compounds with a common core structure by identifying the desired core structure, identifying an MCCA reaction capable of generating that core structure, followed by preparing an array of compounds using the identified MCCA reaction according to the aforementioned method. The present invention also relates to a method for conducting *in vitro* assays of biological material using the combinatorial arrays of the present invention.

SYNTHESIS OF COMBINATORIAL ARRAYS OF ORGANIC COMPOUNDS THROUGH THE USE OF MULTIPLE COMPONENT COMBINATORIAL ARRAY SYNTHESES

Field of the Invention

The present invention relates to a method for generating arrays of compounds having a common core structure through a combinatorial array synthesis using a multiple component combinatorial array (MCCA)

5　synthesis. The present invention also relates to arrays of organic compounds wherein each array is comprised of a group of structural analogues having a common core structure, the core structure being generated by a multiple component one pot synthesis.

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Background of the Invention

Understanding the relationship between a molecule's structure and its biological function is essential to the development of new and improved

15　therapeutic agents. For example, the contours of a receptor's binding site or an enzyme's active site can be effectively analyzed by testing the binding affinity of several structural analogues to these sites.

In nature, many biologically active molecules

20　possess one of a relatively small group of common core structures. These common core structures include β-lactams, peptides, sugars, nucleosides, aromatics, pyridines, steroids, tetrazoles, pyrazines, terpines and alkaloids. Biological activity is often associated with

25　the presence of one of these core structures. Structure-function studies using a group of compounds that share a common core structure but where the members of the group have differing substituents have been used to elucidate numerous biological mechanisms. Several of

30　these studies and the mechanisms that were determined are cited in Goodman & Gilman's The Pharmacological Basis of Therapeutics, Macmillian (6th Edition, 1980).

In addition to being a valuable research tool for understanding biological mechanisms, structure-

function analysis using substituent and structural variants of a known biologically active molecule is an invaluable tool for developing new therapeutic agents. For example, improved penicillin based antibiotics were

5   developed by mimicking penicillin's β-lactam core structure with a core structure that is not susceptible to degradation by β-lactamase. Many antiviral drugs, such as the A.I.D.S. drug 3-azidothymidine (AZT), are designed to mimic the core structure of nucleosides

10   based on the notion that these nucleoside structural analogues will become incorporated into and interfere with the virus's genetic machinery.

The rate limiting step in the analysis of structure-function relationships is the synthesis of the

15   necessary structural analogues. The traditional strategy for evaluating structure-function relationships involves an iterative process whereby successive groups of compounds, all having a common core structure, are synthesized and assayed. After each iteration, the

20   assay results are evaluated in order to design the next group of compounds to be synthesized and assayed. This process is repeated until the substituents or structural factors influencing biological activity are determined and/or optimal therapeutic agents are identified.

25   The traditional approach to evaluating structure-function relationships is extremely time consuming and labor intensive, often requiring the synthesis of greater than one thousand compounds over the course of several years. The chemical syntheses of

30   these compounds are often complex, generally requiring a multistep synthesis for each compound. As a result, a high degree of skill in the synthetic chemical arts is generally required to synthesize the compounds needed to perform these structure-function studies. This greatly

35   limits the number of scientists that can perform this type of structure-function analysis research. Further, the labor demands for this type of research is very

intense, often requiring the full time efforts of
several organic chemists per project. Because of these
labor and skill demands, performance of this type of
research and hence the development of new therapeutic
5   agents is often limited to larger chemical firms.
Researchers possessing a strong understanding of the
biological system being studied are often prevented from
participating in the development of new therapeutic
agents because of their inability to access the
10   compounds needed for performing these studies.

The labor demands associated with synthesizing
structural analogues has also greatly limited the range
of compounds for which structure-function analysis has
been performed. The synthetic effort required to
15   produce structural analogues has made it impractical to
probe core structures not currently associated with
known biologically active molecules for biological
activity. However, it is likely that the number of core
structures associated with biologically active compounds
20   greatly exceeds the number of structures that have been
identified. A more efficient method for synthesizing
structural analogues would make it feasible to evaluate
a far greater range of structures for biological
activity.

25   In contrast to the effort required to
synthesize compounds, the effort required to screen the
synthesized compounds for biological activity is
relatively low. A simple and efficient method for
synthesizing compounds useful for probing for biological
30   activity would significantly accelerate the rate of new
drug development and enable a greater number of
scientists to participate in the research.

One of the factors that makes the synthesis of
compounds used in structure-function research studies so
35   time consuming and research intensive is the fact that
the chemical syntheses of these compounds are performed
separately through stepwise linear transformations.

Since the time required to screen compounds is
significantly smaller than the time required to
synthesize the compounds, it would be advantageous from
a time management standpoint to synthesize more
5 compounds by a more rapid method, even if the total
number of compounds that would have to be screened were
increased.

The general approach for screening monoclonal
antibodies to a specific antigen presents a useful model
10 for how structure-function studies should be conducted.
After antigen stimulation of a host, the monoclonal
antibody producing B-cells of the host are immortalized
and diluted over a large array of microtiter wells.
Most of the monoclonal antibodies produced by the
15 individual immortalized cells in each titer well have
little utility. However, assays exist to identify the
useful antigen binding antibodies in the array.
Similarly, it is possible to rapidly assay large arrays
of organic compounds for biological activity.
20 Unfortunately, however, there are no known biological
systems that can be stimulated to produce an array of
organic chemicals from which a desired compound can be
isolated. Thus, the need exists for a simple and
efficient method for generating an array of organic
25 compounds from which biologically active agents can be
identified.

The methodology developed for the automated
synthesis of biopolymers such as RNA, DNA, polypeptides
and most recently oligosaccharides also presents a
30 useful model for how structure-function studies could be
conducted. The automated synthesis of these biopolymers
has greatly accelerated the development of the
biotechnology arts, primarily because RNA and DNA
probes, as well as small polypeptides, are readily
35 obtainable by all researchers. The benefits derived
from automated biopolymer synthesis technology
highlights the current need for a simple, efficient and

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rapid method for generating numerous organic compounds
to probe structure-function relations in route to the
development of new therapeutic agents.

The automated synthesis of these biopolymers
is achieved by performing a repetitive series of
reactions on the biopolymer which is attached to a solid
support. Unfortunately, biopolymer synthesis technology
cannot be readily redirected to the production of
structural analogues of organic compounds. Unlike
biopolymer synthesis, the synthesis of structural
analogues of organic compounds involves numerous
different chemical reactions. Also, because of the
wider variety of chemical reactions and reagents
involved, a wider variety of solid phase supports and
chemical linkers are needed.

SUMMARY OF THE INVENTION

The present invention relates to an array of
compounds having a common core structure wherein the
compounds of the array comprise the products of a
multiple component combinatorial array synthesis having
at least three reactive components. Each component of
the multiple component combinatorial array synthesis
comprises a group of reactants having a common
functional group. The array synthesis is conducted
under appropriate conditions such that the common
functional group of each component in the reaction
reacts with functional groups on the other components to
form an array of compounds having a common core
structure. Each component of the combinatorial array
synthesis can itself comprise an array of compounds
having a common core structure, the array of component
reactants being synthesized by a multiple component
combinatorial array synthesis. Each component can also
comprise a group of reactants wherein each reactant
comprising a mixture of compounds having a common
functional group. In a further embodiment of the

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present invention, the array of compounds is formed
while bound to a solid support.

The present invention also relates to a method
of making an array of compounds having a common core
5   structure using a multiple component combinatorial array
synthesis with at least three components. Each
component comprises a group of reactants having a common
functional group. The method comprises organizing a
series of reaction vessels in an n dimensional array
10 wherein each reaction vessel is identifiable by its
coordinates in the n dimensional array. Each axis in
the n dimensional array corresponds to a different
component in the array synthesis. Each position on each
axis corresponds to a different reactant of the
15 corresponding component. The reactants of the n
components are added to the n dimensional array of
reaction vessels such that the same reactant is added to
all of the reaction vessels in the array having a
position on the array corresponding to that reactant.
20 The components in each reaction vessel are then reacted
under appropriate conditions to form the compounds of
the array. Each component of the combinatorial array
synthesis can itself comprise an array of compounds
having a common core structure, the array of component
25 reactants being synthesized by a multiple component
combinatorial array synthesis. Each component can also
comprise a group of reactants, each reactant comprising
a mixture of compounds having a common functional group.
A further embodiment of the present invention relates to
30 solid phase multiple component array synthesis which
comprises the further step of binding one of the
components to a solid support.

The present invention also relates to a method
of creating a combinatorial array of compounds with a
35 common core structure by identifying the desired core
structure, identifying a MCCA reaction capable of
generating that core structure, followed by preparing an

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array of compounds using the identified MCCA reaction
according to the aforementioned method.

The present invention also relates to a method
for conducting _in vitro_ assays of biological material by
5   adding biological material to an array of compounds,
each member compound of the array having a common core
structure and being bound to a solid support followed by
measuring the effect each member compound of the array
has on the biological material's biological activity.
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BRIEF DESCRIPTION OF THE FIGURES

Figure 1a-p provides a summary of some of the
common core structures synthetically accessible by MCCA
reactions.

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Figure 2 depicts the synthesis of the core
structures depicted in Figure 1, showing the
correspondence between the substituents (R, R_1, R_2, R_3,
R_4, etc.) in the product and in the reaction components.
20   Figure 2a depicts a synthesis described in Joucia, et
al., _J. Tetrahedron Lett._, (1985) _26_:1221. Figure 2b
depicts a synthesis described in Kelly, et al., _J. Am._
Chem. Soc., (1985) _107_:3879. Figure 2c depicts a
synthesis described in Banville, et al. _Can. J. Chem._,
25   (1974) _50_:80; Cameron, et al., _J. Chem. Soc. Chem._
Commun., (1976) 275; and Cameron, et al., _J. Chem. Soc._
Chem. Commun., (1977) 297. Figure 2d depicts a
synthesis described in Kelly, et al., _J. Amer. Chem._
Soc. (1985) _107_:4998. Figure 2e depicts a synthesis
30   described in Posner, et al., _Tetrahedron_ (1981) _37_:3921.
Figure 2f depicts a synthesis described in Davis, et
al., _J. Org. Chem._, (1979) _44_:3755. Figure 2g depicts a
synthesis described in Bestmann, et al., _Angew Chem._
Int. Ed. Engl, (1985) _24_:790. Figure 2h depicts a
35   synthesis described in Weis, et al., _Tetrahedron Lett._,
(1981) _22_:1453. Figure 2i depicts a synthesis described
in Ugi, et al., _Justus Liegis Ann. Chem._, (1967) _709_:1.

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Figure 2j depicts a synthesis described in Ugi, et al.,
Chem. Ber. (1961) 64:734. Figure 2k depicts a synthesis
described in Sebti, et al., _Synthesis_ (1983) 546.
Figure 2l depicts a synthesis described in Isenring, et
5   al., _Synthesis_ (1981) 385 and Isnering, et al.,
Tetrahedron (1983) 39:2591. Figure 2m depicts a
synthesis described in Kunz, et al., _J. Amer. Chem. Soc._
(1988) 110:651. Figure 2n depicts a synthesis described
in Boehm, et al., _J. Org. Chem._ (1986) 51:2307. Figure
10   2o depicts a synthesis described in Murakami, et al., _J._
Org. Achem., (1993) 58:1458. Figure 2p depicts a
synthesis of a c-linked disaccharide wherein the
stereochemistry of the first ring is determined by the
sugar used in the synthesis and the stereochemistry of
15   the second ring is determined by the oxidation catalyst.

Figure 3 depicts the fact that MCCA array
syntheses produce analogues on a geometric scale where
the total number of analogues synthesized equals the
20   product of the number of structural variants of each
component of the MCCA array synthesis used.

Figure 4 depicts the results of a two
dimensional MCCA array synthesis using the Passerini
25   reaction wherein the aldehyde and acid reaction
components are varied.

Figure 5 depicts the mechanism of action for
carzinophilin/azinomycin.
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Figure 6 depicts the α-acyloxy amine core
structure of the antitumor antibiotic
carzinophilin/azinomycin. By labelling the substituents
off of the α-acyloxy amine core structure R_1, R_2 and R_3,
35   a Passerini reaction scheme for the synthesis of
carzinophilin/azinomycin and its derivatives is
identified.

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Figure 7 depicts the Ugi reaction which, upon removal of the protecting groups, provides nonhydrolizable peptide analogues that mimic a natural peptide's structure.

Figure 8 depicts the peptide backbone core structure of phosphotyrosine peptides and the synthesis of structural variants of phosphotyrosine by varying the first and second amino acids employed (aa1 and aa2) as well as the other components of the Ugi reaction.

Figure 9 depicts the synthesis of a series of tyrosine-based peptide analogues. Figure 9a depicts the synthesis of pseudosubstrate peptide inhibitors. Figure 9b depicts the synthesis of low molecular weight tyrosine-based peptide analogues where the tyrosine structural unit is intact. Figure 9c depicts the synthesis of low molecular weight tyrosine-based peptide analogues where the tyrosine structural unit is modified. Figure 9d depicts the synthesis of additional low molecular weight tyrosine-based peptide analogues.

Figure 10 depicts the synthesis of a support and linker system.

Figure 11 depicts the solid phase synthesis of peptidomimetics by means of the Ugi reaction.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a simple and efficient method for synthesizing arrays of organic compounds having a common core structure. More specifically, the present invention relates to the use of multiple component combinatorial array (MCCA) syntheses to synthesize arrays of structurally related analogues having a common core structure.

Multiple component combinatorial array (MCCA)
syntheses correspond to reactions where the reactants
combine synchronously or asynchronously in one reaction
vessel to form the product. Over the years, numerous
5 MCCA reactions have been developed. In some cases, the
reactions were developed in order to show an efficient
synthesis of a known natural product. Cameron, et al.,
J. Chem. Soc. Chem. Comm., (1976) 275 (synthesis of m-
deoxygenated benzoquinones in the application of the
10 total synthesis if anthraquinone insect pigments);
Schopf, Angew. Chem., (1937) 50:779-797 (double Mannich
reaction in the total synthesis of tropane alkaloids);
Posner, et al., Tetrahedron, (1981) 39:3921. In other
cases, the reactions were developed as a general entry
15 into methodology involving the formation of multiple
bonds in a cascade process. In any event, these
reactions represent a simple and efficient means for
generating numerous structural variants sharing the core
structure generated by the given MCCA reaction. A
20 summary of some of the core structures that are made
synthetically accessible by MCCA reactions is provided
in Figure 1. The reaction sequence for generating these
core structures is summarized in Figure 2.

Traditionally, structural analogues have been
25 synthesized by multiple step linear syntheses. Linear
syntheses involve the sequential reaction of several
separate reactants in order to achieve the final
product. Linear syntheses are generally not one pot
reactions, requiring the isolation and purification of
30 intermediate products. Unlike linear syntheses, MCCA
reactions, because they are one pot syntheses, do not
require the isolation and purification of intermediate
reaction products. As a result, MCCA reactions are
simpler and more efficient to perform than multiple step
35 linear syntheses. Products of MCCA reactions are
therefore more accessible to those lacking a high level
of skill in the synthetic organic chemical arts.

Further, since no intermediate isolation and
purification is required, MCCA reactions are more
readily adaptable to automation.

5   MCCA reactions, when used in the form of an
array synthesis, enable the synthesis of structural
analogues on a geometric rather than linear scale.   In
an MCCA array synthesis, at least three reaction
components are used. Each component comprises a group
of reactants possessing a common functional group that

10 participates in the MCCA reaction. The combinatorial
array synthesis is conducted by reacting the different
combinations of the various MCCA components.
Combinatorial array syntheses are depicted in Figures 3,
7 and 9.

15   A MCCA array synthesis can be characterized by
the number of reaction components involved and the
number of variants of each reaction component employed.
In a MCCA array synthesis where the MCCA reaction has n
components, an n dimensional array of structural

20 analogues can be produced. Each array of analogues is
equivalent to a library of analogues. When one reactant
is held constant (or when a component is comprised of a
group of one reactant), the n dimensional array
simplifies to an n-1 array and can be visualized as a

25 sublibrary of a greater array where the reactant used
for that component is held constant.

             As depicted in Figure 3, an MCCA array
synthesis is analogous to the multiplication of a series
of one dimensional arrays. Each component of the array

30 synthesis is represented by a different one dimensional
array, the elements of the one dimensional array
corresponding to the group of reactants used for a given
component in the combinatorial array synthesis.
Mathematically, an n dimensional array is generated when

35 n one dimensional arrays are multiplied. In the case of
an MCCA array synthesis, the reaction vessels are
organized in an n dimensional array wherein each

reaction vessel is identifiable by its coordinates in an n dimensional array. Each axis of the array of reaction vessels corresponds to a different component in the MCCA reaction. Each position on each axis corresponds to a

5   different reactant of the corresponding component. During the combinatorial array synthesis, the reactants of the n components are added to the n dimensional array of reaction vessels such that the same reactant is added to all of the reaction vessels in the array having a

10 position on the array corresponding to that reactant. The reaction of the components in the array of reaction vessels is analogous to the multiplication of the n one dimensional arrays since the reaction of the various reactants in each reaction vessel in the n dimensional

15 array results in the "product" of the reactants added to that reaction vessel.

        MCCA array syntheses have the advantage over linear syntheses in that they produce analogues on a geometric scale rather than a linear scale. The ability

20 to synthesize structural analogues on a geometric scale significantly decreases the time and effort required to synthesize these compounds.

        As depicted in Table 1, the total number of analogues synthesized equals the product of the number

25 of each reactant employed. For example, if 10 different variants of each component of a three component MCCA are employed, a 10x10x10 three dimensional array of analogues is produced which corresponds to an array of 1000 structural analogues.

30

TABLE 1

No. of Components	No. of Reactants Per Component	No. of Analogues
3	3	27
3	5	125
3	10	1000
4	5	625
5	5	3125

The combinatorial array synthesis described above provides a wide variety of structural analogues limited only be the reactive limits of the underlying MCCA reaction. For example, in a further embodiment of the present invention, the reactants of a component of a combinatorial array synthesis can themselves be the product of a combinatorial array synthesis. In yet a further embodiment of the present invention, individual reactants of a component of an MCCA array synthesis can comprise mixtures of different compounds having a common functional group. When the individual reactants used comprise mixtures of compounds, the analogues produced by the synthesis are a mixture of compounds. Hence, the term "analogue," as it is used in this application, can include more than one compound. Further, depending on the particular reaction, cis, trans, exo, endo and diasteriometric isomers can also be produced by the array synthesis.

It should be understood that the MCCA array syntheses of the present invention encompass all type of chemical reactions including, but not limited to solution, solid phase, photochemical, electrochemical, free radical and enzymatic reactions.

It should also be understood that MCCA array syntheses involving the condensation of four or more components can also be conducted according to the present invention. In the case of a four component array synthesis, it is simplest to view the reaction as a series of three dimensional (n-1) array syntheses where the only variable between the three dimensional

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arrays is the structure of the fourth component used in each array. Higher order MCCA array syntheses are also possible.

5   The present invention also relates to the arrays (libraries) of structural analogues that can be synthesized by an MCCA array synthesis. Assaying compounds requires very little time relative to the time presently required to synthesize compounds by linear syntheses. The present invention enables the rapid

10   production of large libraries of structurally related analogues all having a common core structure. Given the speed with which compounds can be analyzed, access to libraries of compounds sharing a common core structure would be an invaluable tool for analyzing the structure-

15   function relationships governing a molecule's biological activity.

At present, a relatively small group of core structures appear to predominate in nature. Reference arrays of structural analogues of these common core

20   structures would be valuable for making at least preliminary screenings regarding which core structures and which analogues having those core structure arrays possess some biological activity. Although these reference arrays may not always be specifically designed

25   for the biological system being tested, the observation of some change in biological activity with regard to certain analogues within the arrays would provide valuable clues as to the governing structure-function relations. These clues could then be used to design

30   more specifically tailored combinatorial arrays and/or specific target compounds.

Access to combinatorial arrays of structural analogues of core structures found in nature would also be invaluable for expeditiously performing preliminary

35   structure-function analyses on newly isolated biomolecules that have not been fully characterized regarding their biological function. Further, these

combinatorial arrays would provide smaller research
groups and, in particular, non-synthetic research
groups, with access to compounds for doing structure-
function analyses that were previously inaccessible.

5   The ability of the present invention to
generate large quantities of structural analogues now
makes it feasible, for the first time, to investigate
the biological activity of arrays of compounds with core
structures not currently associated with biologically

10 active molecules. Previously, the synthetic effort
required to produce structural analogues made it
impractical to probe core structures not associated with
known biologically active molecules for biological
activity. The large arrays of structural analogues that

15 can be generated by the present invention now make it
feasible to assay a wider range of core structures and
structural analogues of these core structures for
biological activity.

          A very small amount of material is needed to
20 assay a compound for the presence of biological
activity. Therefore, combinatorial arrays of structural
analogues would not need to contain large amounts of any
given compound. In addition, since a small quantity of
compound is needed per assay, even combinatorial arrays
25 containing small quantities of each compound would be
capable of repeated use.

          The organizational sense of the combinatorial
arrays of the present invention greatly simplifies the
mental aspect of structure-function analysis. In order
30 to appreciate this utility, the organizational nature of
the combinatorial arrays of the present invention needs
to be explained. The combinatorial array of compounds
generated by a MCCA synthesis is best thought of in
terms of a geometric array having a dimension equal to
35 the number of reaction components employed in the MCCA
array synthesis. For example, a four component MCCA
reaction would generate a four dimensional combinatorial

array. Each element of the array is identifiable by its coordinate in the array which corresponds to the precise components used to synthesize that analogue. Hence the product of the second variant of each component of a
5 three dimensional array would have an array coordinate of (2,2,2).

The coordinate system of the combinatorial array assists the evaluation of results derived from assaying the elements of a particular combinatorial
10 array for biological activity. Each n dimensional array can be envisioned as a series of (n-1) dimensional arrays or sublibraries wherein all of the members of each sublibrary share a common core structure formed by at least one common component. Thus, as depicted in
15 Figure 3, a three component condensation reaction can be broken down into a series of two dimensional arrays where one reactant is held constant.

The formation or destruction of activity across a sublibrary reveals the biological significance
20 of the structure contributed by the component common to all of the members of the sublibrary. Thus, by interpreting MCCA arrays as multidimensional arrays that can be divided into sublibraries, the present invention provides a systematic framework for evaluating
25 structure-function relationships. Since each sublibrary, independent of its size, corresponds to a class of compounds where one structural subunit is held constant, a finding of biological activity across a particular sublibrary would indicate that the unvaried
30 functional subunit was significant to the biological activity realized for that group of compounds.

The organizational sense created by the combinatorial arrays of the present invention provide an invaluable tool for evaluating the structure-function
35 correlations of a very large number of compounds. The linear synthetic approach currently employed for the identification of new therapeutics is driven largely by

evaluating the binding constants of the molecules
assayed. The rational as to why certain analogues
exhibit stronger binding is not necessarily readily
apparent from those studies. In contrast, the approach
5   of the present invention provides an organizational
framework whereby structure-function effects become
readily apparent. Thus, in addition to providing a more
rapid and efficient route to analogues for testing
structure-function relationships, the present invention
10 provides a more effective means for evaluating
structure-function relationships in route to the
identification of superior therapeutic agents.

The present invention also relates to the
solid phase synthesis, storage and use of these MCCA
15 arrays. Solid phase synthesis is valuable for
simplifying purification. By binding one of the
reaction components to a solid support, for example, a
microtiter well, it is possible to isolate the compounds
synthesized in high purity. One of the disadvantages of
20 any multiple step reaction is low yields. A three step
synthesis having a 70% yield for each step only produces
a product in a 35% yield. Thus, when synthesizing
complex molecules, simplification of the purification
process is highly desirable as a means for optimizing
25 the resulting yield. By synthesizing the compounds of
the combinatorial array on a solid support where one of
the reactants is attached to a detachable linker,
purification and isolation of the product is greatly
simplified. This is especially important when complex,
30 lower yielding MCCA reactions are involved since all
that is needed in the present invention is an adequate
amount of each analogue to test for biological activity.

Solid phase synthesis of the combinatorial
arrays of the present invention improves the storage
35 stability of these arrays by enabling these compounds to
be isolated in high purity and in a solid state. In
addition, storing the compounds while bound to a solid

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support prevents the spillage or mixing of the compounds
in the combinatorial array.

        Solid phase synthesis also facilitates the use
of these combinatorial arrays for conducting _in vitro_
5   binding studies. Entire two dimensional arrays of
compounds bound to microtiter trays can be assayed at
one time for their ability to bind to a protein or cell.
This enables entire arrays of analogues to be very
rapidly assayed for binding to different biomolecules
10 and cells.

        Solid phase synthesis of combinatorial arrays
by MCCA reactions presents very few limitations on the
different types of compounds that can be synthesized.
In general, the only limitation presented by the use of
15 solid phase synthesis is the requirement that there be a
means for attaching one of the reaction components to
the solid support. This will generally involve the use
of a functional group such as an alcohol, mercaptan,
amine, carbonyl, carboxylate or selenide. No other
20 limitations to the structure of the compound to be
synthesized are created. Further, as new linkers are
designed, the structural limitations presented will be
further reduced.

        Any solid support media capable of covalently
25 binding to a linker can be used in the present
invention. Polymers such as polystyrene-divinyl
benzene, polyethylene grafted polystyrene,
polyacrylamide-kieselguhr composites and controlled pore
glass have all been commercialized with functional
30 groups suitable for derivitization with various linkers.

        Linkers are used to couple a variety of
monomers to the solid support for the solid phase
polymeric synthesis of biomolecules such as DNA and
peptides. The linkers of the present invention must be
35 capable of binding to one of the components of the MCCA
synthesis and must also be capable of later releasing
the synthesized molecules by some specific, regulatable

mechanism. Such regulatable methods include but are not limited to thermal, photochemical, electrochemical, acid, base, oxidation and reduction reactions.

There are several commercially available
5   linkers which provide a variety of functional group coupling and cleavage strategies. Examples of acid labile linkers include the 4-hydroxy methyl phenoxy aliphatic acids which allow the coupling of ethers acetals and esters to the linker. Acid labile linkers
10 bearing an amine, such as the p-[2,4-dialkoxylaminobenzyl] phenoxy acetic acid, allow for coupling through functional groups such as amides, imines, carbonates or ureas. The extent of the alkoxyl substituents on the aromatic rings of these linkers
15 influence the pH at which the organic compound is cleaved from the solid support. Trityl-based linkers can be cleaved under mildly acid conditions.

Base labile linkers include [4-(2-bromopropionyl)phenoxy] acetic acid linkers. The ester
20 coupling of these linkers can be cleaved by nucleophiles such as hydroxide, alkoxides, and amines to give the corresponding acid, ester and amide derivatives of the organic compounds. In addition, the [4-(2-bromopropionyl)phenoxy] acetic acid linker as well as
25 the ortho-nitro benzyl linkers enable the photochemical cleavage of an organic compound from solid support under neutral conditions.

It is preferred that the linker be capable of photochemically or thermally releasing the product since
30 no decoupling reactants are required. A linker having a thermal release mechanism is the most preferred embodiment because thermal reactions provide greater control over the rate at which the compound is released. Further, thermally labile linkers are more stable than
35 photochemically labile linkers.

The types of linkers useful in the present invention is not limited to the linkers described herein

since additional suitable linkers may exist in the art
or be later developed. The use of linkers in the solid
phase synthesis of an MCCA array is described in
Examples 6 and 7.

5   The present invention also relates to the use
of solid support bound analogues in _in vitro_ binding
studies. In this application of the present invention,
whole arrays of compounds can be simultaneously, quickly
and efficiently tested for their ability to bind to a
10   biological molecule. This greatly accelerates the
process of assaying large arrays of compounds for
biological activity.

          Arrays of compounds bound to a solid support
can also be used to study the effect of structural
15   orientation on a compound's biological activity. This
is accomplished by assaying combinatorial arrays of the
same group of compounds where each array differs in that
a different portion of the structural analogues in each
array are used to bind the compounds to the solid
20   support. Hence, the present invention provides a method
for testing the binding affinity of different faces of
the same array of compounds. The linear synthesis
approach currently employed does not provide a means for
obtaining this data short of obtaining crystal
25   structures of bound complexes.

          One of the limitations of using solid phase
synthesis is the requirement that one of the components
be attachable to the solid support and also be able to
participate in the MCCA reaction. In order for a
30   component to serve both these functions, the component
must have two functional groups, one that participates
in the reaction and the other which binds to the linker.
The requirement that a component have two functional
groups is not a significant problem when one does not
35   seek to vary the structure of the component being bound
to the solid support. However, the range of structural
variants of a component that can be both bound to a

linker and at the same time participate in the reaction
is often limited.

It should be noted that it is not always
necessary to isolate and purify the products of an array
5   synthesis. Many MCCA reactions are high yielding and
all components can be reacted in solution. Further,
many bioassays are operable on crude product mixtures.
Hence, in these instances, the primary advantage of
solid phase synthesis, namely simplified purification,
10   is obviated.

In addition, a method for isolating reaction
products from solution chemistry reaction mixtures
involves sequestering the unreacted components of the
reaction. An example of the use of sequestering agents
15   to isolate the products of an Ugi reaction is provided
in Example 9. By using sequestering agents to sequester
the unreacted components of the reaction, the products
of the array synthesis can be isolated at sufficient
purity to use in bioassays.

20   The present invention also relates to a method
for the automated production of structural analogues.
The automated synthesis of MCCA arrays reduces the level
of skill required for the preparation of arrays of
structural analogues and thus enables the performance of
25   structure-function studies without the assistance of
synthetic organic chemists. Thus, much like automated
DNA, RNA and peptide synthesizers, the present invention
enables researchers without a high level of skill in the
synthetic chemical arts to generate combinatorial arrays
30   of structural analogues. The automated synthesis of the
combinatorial arrays of structural analogues of the
present invention is made possible by the logical
organization of the arrays synthesized. Automation of
the present invention is facilitated by the fact that
35   the present invention can be organized so that the same
reaction component can be added to all of the reaction
compartments in the same row, column or layer.

22

Automated synthesis is also facilitated by the fact that the same MCCA reaction conditions can be used for the synthesis of all of the compounds within the MCCA array.

Automated combinatorial synthesis of MCCA
5   arrays can be performed in both the solution phase as well as in the solid phase. In many cases, the MCCA reactions are high yielding such that the major component of the reaction mixture is the product. In such cases, the product can often be isolated by
10   evaporation of the solvent and other volatiles.

In the case of lower yielding MCCA reactions, the automated combinatorial synthesis of MCCA arrays is preferably conducted in the solid phase because the purification and isolation of the synthesized compounds
15   is greatly simplified.

The method of generating combinatorial arrays of structural analogues using multiple component condensation reactions as well as the combinatorial arrays generated are illustrated in the following
20   examples. As is shown by these examples, the present invention, through the use of MCCA reactions, provides a simple and efficient method for synthesizing large numbers of structural analogues for most of the known common core structures of biologically active molecules.
25   Further objectives and advantages other than those set forth above will become apparent from the examples and accompanying drawings.

It should be noted that some reactions may not function within the disclosed scope of the invention.
30   The compounds for which this occurs will be readily recognized by those skilled in the art. In all such cases, either the reactions can be successfully performed by conventional modifications known to those skilled in the art. e.g., by appropriate protection of
35   interfering groups, by changing to alternative conventional reactants, or by routine modification of reaction conditions. Alternatively, other reactions

disclosed herein or otherwise conventional will be
applicable to the preparation of the corresponding
compounds of the invention. In all preparative methods,
all starting materials are known or readily preparable
5   from known starting materials; all temperatures are set
forth uncorrected in degrees Celsius; and, unless
otherwise indicated, all parts and percentages are by
weight.

24

EXAMPLES

EXAMPLE 1 <u>Determination of the MCCA Array To Prepare</u>
The present invention takes advantage of the

5   organic chemistry synthesis design theory known as the
retrosynthetic approach. The retrosynthetic approach
teaches one to dissect a target molecule into its
smaller, simpler components by looking for bonds in the
molecule that can be formed by known synthetic methods.

10   The present invention takes an analogous approach,
teaching one to evaluate the target molecule for the
presence of one or more of the core structures known to
be accessible by MCCA reactions.

In order to determine which MCCA reaction to

15   employ to generate a series of structural analogues of
the target molecule, it is first necessary to compare
the target compound with the core structures generated
by MCCA reactions. Figure 1 summarizes fourteen core
structures that can be generated by known MCCA

20   reactions. Once a MCCA core structure in the target
molecule is identified, the scheme for synthesizing the
target molecule is determined by labelling the
substituents attached to the core structure as R, R_1, R_2,
R_3, etc. as indicated. Then, as shown in Figure 2, the

25   same substituents are attached to the corresponding
reaction components. A combinatorial array of
structural analogues of the target molecule can be
prepared by varying the structure of the substituents of
the various components of the MCCA reaction. This

30   procedure is further described in Example 3 which
describes the synthesis of carzinophilin/azinomycin
structural analogues using a Passerini MCCA reaction.

EXAMPLE 2 Synthesis of α-Acyloxy Amine MCCA
35   <u>Arrays Using The Passerini Reaction</u>

The synthesis of an array of structural analogues
having a common α-acyloxy amine core structure can be

prepared by a combinatorial array synthesis using the
Passerini reaction. As depicted in Figure 3, the
Passerini reaction involves the reaction of an aldehyde
having the general structure RCHO with an isocyanide
5   having the general structure R_1NC and an acid having a
general structure R_2COOH.

A two-dimensional MCCA array synthesis
employing the Passerini reaction was conducted using
eight aldehydes, eight carboxylic acids and one
10 isocyanide. The two dimensional combinatorial array of
analogues generated by this synthesis, along with the
observed yields, is depicted in Figure 4. The
experimental protocol for this combinatorial array
synthesis as well as the physical data for ten of the
15 analogues synthesized is provided below. All of the
reactants used in the two dimensional array synthesis
are commercially available.

Experimental Protocol:

20

A.   Reactant Preparation:

An anhydrous CH_2Cl_2 (17 mL) solution of diethyl
cyanomethylphosphonate (700 mg, 70 equiv.) was prepared
in a 25 mL round bottom flask and allowed to stir for 1
25 hour at room temperature under an N_2 atmosphere.
Anhydrous CH_2Cl_2 (2.2 mL) solutions of aldehydes:
benzaldehyde, heptaldehyde, propanaldehyde, trans-2-
butenal, p-methoxybenzaldehyde, butyraldehyde, 4-N,N-
dimethylaminobenzaldehyde, and cinnamaldehyde were
30 prepared in 5 mL round bottom flasks such that each
aldehyde represented 20 molar equivalents based on
isocyanide. An anhydrous CH_2Cl_2 (2.2 mL) solution of
carboxylic acids: acetic acid, 2-phenylacetic acid,
2',2-diphenylacetic acid, acrylic acid, benzoic acid, 1-
35 naphthoic acid, cinnamic acid, 3,3-dichloropropionic
acid were prepared in 5 mL round bottom flasks such that

each acid represented 10 molar equivalents based on isocyanide.

B.   Reaction Protocol:

A shell vial box (Fisher, 1 dr, 15 X 45 mm, 72 vials) containing 64 glass vials (8X8) was used as the reaction chamber for the combinatorial array synthesis. The specific aldehyde used in the combinatorial array synthesis was varied by rows. The specific acid used in the combinatorial array synthesis was varied by columns.

Row 1:

Using a gas tight syringe, 2.2 mL CH_2Cl_2 solution of cinnamaldehyde in 0.25 mL (1 equiv.) aliquots were added into each of eight vials in row 1 in one single process.

Row 2:

Using a gas tight syringe, 2.2 mL CH_2Cl_2 solution of 4-N,N-dimethylbenzaldehyde in 0.25 mL (1 equiv.) aliquots were added into each of eight vials in row 2 in one single process.

Row 3:

Using a gas tight syringe, 2.2 mL CH_2Cl_2 solution of butyraldehyde in 0.25 mL (1 equiv.) aliquots were added into each of eight vials in row 3 in one single process.

Row 4:

Using a gas tight syringe, 2.2 mL CH_2Cl_2 solution of p-methoxybenzaldehyde in 0.25 mL (1 equiv.) aliquots were added into each of eight vials in row 4 in one single process.

Row 5:

Using a gas tight syringe, 2.2 mL CH_2Cl_2 solution of trans-2-butenal in 0.25 mL (1 equiv.) aliquots were added into each of eight vials in row 5 in one single process.

Row 6:

Using a gas tight syringe, 2.2 mL CH_2Cl_2
solution of propanaldehyde in 0.25 mL (1 equiv.)
aliquots were added into each of eight vials in row 6 in

5 one single process.
Row 7:

Using a gas tight syringe, 2.2 mL CH_2Cl_2
solution of heptaldehyde in 0.25 mL (1 equiv.) aliquots
were added into each of eight vials in row 7 in one

10 single process.
Row 8:

Using a gas tight syringe, 2.2 mL CH_2Cl_2
solution of benzaldehyde in 0.25 mL (1 equiv.) aliquots
were added into each of eight vials in row 8 in one

15 single process.
Column A:

Using a gas tight syringe, 2.2 mL CH_2Cl_2
solution of acetic acid in 0.25 mL (1 equiv.) aliquots
were added into each of eight vials in column A in one

20 single process.
Column B:

Using a gas tight syringe, 2.2 mL CH_2Cl_2
solution of 2-phenylacetic acid in 0.25 mL (1 equiv.)
aliquots were added into each of eight vials in column B

25 in one single process.
Column C:

Using a gas tight syringe, 2.2 mL CH_2Cl_2
solution of 2,2-diphenylacetic acid in 0.25 mL (1
equiv.) aliquots were added into each of eight vials in

30 column C in one single process.
Column D:

Using a gas tight syringe, 2.2 mL CH_2Cl_2
solution of acrylic acid in 0.25 mL (1 equiv.) aliquots
were added into each of eight vials in column D in one

35 single process.

Column E:

   Using a gas tight syringe, 2.2 mL CH$_2$Cl$_2$
solution of benzoic acid in 0.25 mL (1 equiv.) aliquots
were added into each of eight vials in column E in one
5 single process.

Column F:

   Using a gas tight syringe, 2.2 mL CH$_2$Cl$_2$
solution of 1-naphthoic acid in 0.25 mL (1 equiv.)
aliquots were added into each of eight vials in column F
10 in one single process.

Column G:

   Using a gas tight syringe, 2.2 mL CH$_2$Cl$_2$
solution of cinnamic acid in 0.25 mL (1 equiv.) aliquots
were added into each of eight vials in column G in one
15 single process.

Column H:

   Using a gas tight syringe, 2.2 mL CH$_2$Cl$_2$
solution of 3,3-dichloropropionic acid in 0.25 mL (1
equiv.) aliquots were added into each of eight vials in
20 column H in one single process.

   The 17 mL CH$_2$Cl$_2$ solution of
cyanomethylphosphonate was dispensed in 0.25 mL aliquots
into 64 vials of the array using a gas tight syringe in
a single process. Upon completion of reactant additions,
25 each reaction vessel contained 2 molar equivalents of
aldehyde, one molar equivalent of acid, and one molar
equivalent of isocyanide in a total volume of 0.75 mL in
CH$_2$Cl$_2$. The reaction vessels (vials) were all capped and
no further precautions were taken to maintain an
30 anhydrous atmosphere. The entire array was manually
shaken for 30 seconds and then allowed to stand at room
temperature for 17 hours. Selected samples were each
transferred to round bottom flasks and evaporated to
dryness under reduced pressure without further
35 purification. Each crude mixture was analyzed by ^{1}H NMR
to determine the product composition and yield based on
unreacted starting isocyanide. Data is provided below

for a selection of ten analogues. Each analogue is
identified by its row and column number.

<u>Analogue 3C</u>:   N-[(1-diethylphosphono)methyl]-2-
5   diphenylacetoylpentamide: This reaction mixture
contained n-butanaldehyde (80 uL, 2 equiv.),
diphenylacetic acid 100 mg, 1 equiv.) and
diethylcyanomethyl phosphonate (10 mg, 1 equiv) ^{1}H NMR
(CDCl$_3$) δ = 7.3 (m, Ar-H), 5.2 (t, OCH), 4.1 (m,
10   OCH$_2$CH$_3$), 3.7 (m, CH$_2$P), 3.5 (m, CH$_2$P), 1.7 (m, CH$_2$), 1.3
(m, OCH$_2$CH$_3$), 1.3 (m, CH$_2$), 0.8 (t, CH$_3$). 80% yield.

<u>Analogue 3F</u>:   N-[(1-diethylphosphono)methyl]-2-(1-
napthoyl)pentamide: This reaction mixture contained n-
15   butanaldehyde (80 uL, 2 equiv.), 1-napthoic acid 80 mg,
1 equiv.) and diethylcyanomethyl phosphonate (10 mg, 1
equiv.), ^{1}H NMR (CDCl$_3$) δ = 9.0 (d, Ar-H), 8.2 (d, Ar-H),
8.0 (d, Ar-H), 7.6 (m, Ar-H), 7.5 (m, Ar-H), 7.3 (Ar-H),
5.5 (t, OC6CH), 4.1 (m, OCH$_2$CH$_3$), 3.8 (m, CH$_2$P), 2.1 (m,
20   CH$_2$), 1.6 (m, CH$_2$), 1.3 (m, OCH$_2$CH$_3$), 1.3 (m, CH$_2$), 1.0
(t, CH$_3$). 80% yield.

<u>Analogue 6C</u>:   N-[(1-diethylphosphono)methyl]-2-
diphenylacetoylbutamide: This reaction mixture
25   contained n-propanaldehyde (70 uL, 2 equiv.),
diphenylacetic acid (100 mg, 1 equiv.) and
diethylcyanomethyl phosphonate (10 mg, 1 equiv.), ^{1}H NMR
(CDCl$_3$) δ = 7.3 (m, Ar-H), 6.6 (bt, NH), 5.2 (t, OCOCH),
5.1 (s, CHAr$_2$), 4.1 (m, OCH$_2$), 3.8 (m, CH$_2$P), 1.85 (m,
30   OCH$_2$CH$_3$), 1.3 (m, OCH$_2$CH$_3$), 1.3 (m, CH$_2$), 0.8 (t, CH$_3$).
60% yield.

<u>Analogue 6F</u>:   N-[(1-diethylphosphono)methyl]-2-(1-
napthoyl)butamide: This reaction mixture contained n-
35   propanaldehyde (70 uL, 2 equiv.), 1-napthoic acid (80
mg, 1 equiv.) and diethylcyanomethyl phosphonate (10 mg,
1 equiv.), ^{1}H NMR (CDCl$_3$) δ = 9.0 (d, Ar-H), 8.2 (d, Ar-

H), 7.8 (m, Ar-H), 7.5 (m, Ar-H), 7.5 (bt, NH), 5.5 (t, OCOCH), 4.1 (m, OCH_2CH_3), 3.85 (m, CH_2P), 2.1 (m, CH_2), 1.4 (m, OCH_2CH_3), 1.3 (m, CH_2), 1.1 (t, CH_3). 75% yield.

5   Analogue 7A: N-[(1-diethylphosphono)methyl]-2-acetoxyoctamide: This reaction mixture contained n-heptaldehyde (125 uL, 2 equiv.), acetic acid (30 mg, 1 equiv.) and diethylcyanomethyl phosphonate (10 mg, 1 equiv.), ^1H NMR ($CDCl_3$) δ = 6.6 (bt, NH), 5.1 (t, OCOCH),
10   4.1 (m, OCH_2CH_3), 3.65 (m, CH_2P), 2.1 (5, CH_3), 1.7 (m, CH_2), 1.55 (m, CH_2), 1.3 (m, OCH_2CH_3), 1.3 (m, CH_2), 0.8 (t, CH_3). 95% yield.

Analogue 7B: N-[(1-diethylphosphono)methyl]-2-
15   phenylacetoyloctamide: This reaction mixture contained n-heptaldehyde (125 uL, 2 equiv.), phenylacetic acid (65 mg, 1 equiv.) and diethylcyanomethyl phosphonate (10 mg, 1 equiv.), ^1H NMR ($CDCl_3$) δ = 7.3 (m, Ar-H), 6.7 (bt, NH),- 5.1 (t, OCOCH), 4.1 (m, OCH_2CH_3), 3.7 (m, CH_2P),
20   3.6 (s, CH_2), 3.55 (m, CH_2P), 1.8 (m, CH_2), 1.3 (m, OCH_2CH_3), 1.3 (m, CH_2), 0.8 (t, CH_3). 50% yield.

Analogue 7D: N-[(1-diethylphosphono)methyl]-2-acyloyloctamide: This reaction mixture contained n-
25   heptaldehyde (125 uL, 2 equiv.), acrylic acid (35 mg, 1 equiv.) and diethylcyanomethyl phosphonate (10 mg, 1 equiv.), ^1H NMR ($CDCl_3$) δ = 6.9 (bt, NH), 6.4 (dd, CH_2CH), 6.1 (dd, CH_2CH), 5.8 (dd, CH_2CH), 5.15 (t, OCOCH), 4.6 (m, OCH_2C11_3), 3.7 (m, CH_2P), 1.8 (OCH_2), 1.5
30   (m, CH_2), 1.3 (m, OCH_2CH_3), 1.3 (m, CH_2), 0.8 (t, CH_3). 100% yield.

Analogue 7E: N-[(1-diethylphosphono)methyl]-2-benzoyloctamide: This reaction mixture contained n-
35   heptaldehyde (125 uL, 2 equiv.), benzoic acid (60 mg, 1 equiv.) and diethylcyanomethyl phosphonate (10 mg, 1 equiv.), ^1H NMR ($CDCl_3$) δ = 8.0 (d, ArH), 7.5 (d, ArH),

7.4 (d, Ar-H), 7.1 (bt, NH), 5.4 (t, OCOCH), 4.1 (m, O-CH$_2$CH$_3$), 3.8 (m, CH$_2$P), 2.0 (m, CH$_2$), 1.6 (m, CH$_2$), 1.3 (m, OCH$_2$CH$_3$), 1.3 (m, CH$_2$), 0.8 (t, CH$_3$). 100% yield.

5   Analogue 7G: N-[(1-diethylphosphono)methyl]-2-cinnamoyloctamide: This reaction mixture contained n-heptaldehyde (125 uL, 2 equiv.), cinnamic acid (70 mg, 1 equiv.) and diethylcyanomethyl phosphonate (10 mg, 1 equiv.), ^{1}H NMR (CDCl$_3$) δ = 7.7 (d, CH), 7.5 (m, Ar-H),
10   7.4 (m, Ar-H), 7.3 (m, Ar-H), 6.5 (d, CH), 5.3 (t, OC6CH), 4.1 (m, 6CH$_2$CH$_3$), 3.8 (m, CH$_2$P), 1.9 (m, CH$_2$), 1.6 (m, CH$_2$), 1.3 (m, OCH$_2$CH$_3$), 1.3 (m, CH$_2$), 0.8 (t, CH$_3$). 70% yield.

15   Analogue 7H: N-[(1-diethylphosphono)methyl]-2-dichloroacetoyloctamide: This reaction mixture contained n-heptaldehyde (125 uL, 2 equiv.), dichloroacetic acid (70 mg, 1 equiv.) and diethylcyanomethyl phosphonate (10 mg, 1 equiv.), ^{1}H NMR
20   (CDCl$_3$) δ = 7.15 (bt, NH), 6.2 (s, CHCl$_2$), 5.3 (t, OCOCH), 4.1 (m, OCH$_2$CH$_3$), 3.7 (m, CH$_2$P), 1.9 (m, CH$_2$), 1.6 (m, CH$_2$), 1.3 (m, OCH$_2$CH$_3$), 1.3 (m, CH$_2$), 0.8 (t, CH$_3$). 95% yield.

25

EXAMPLE 3 Application Of The MCCA Synthesis
           Strategy To Structural Analogues Of
           Carzinophilin/Azinomycin
           Carzinophilin (CZ) and Azinomycin B are
30   antitumor/antibiotic compounds which function as a DNA bis-alkylating agent. The mechanism of action for CZ and Azinomycin B is depicted in Figure 5. As also shown in Figure 5, Carzinophilin and Azinomycin B are structurally very similar. Given the structural
35   complexity of these molecules, chemical syntheses are long and low yielding. Azinomycin B has also been produced by fermentation but in very low yields (2 mg/250 L).
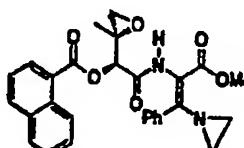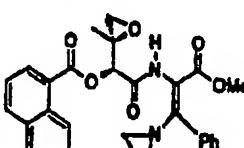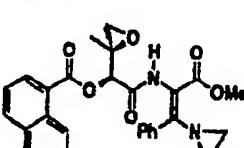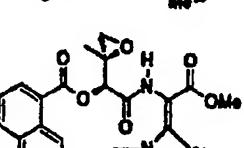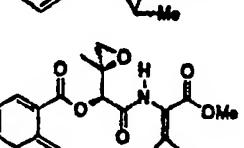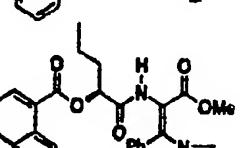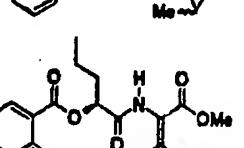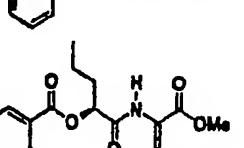
Given the difficulty associated with synthesizing these molecules, few derivatives of these molecules have been prepared. It therefore would be highly desirable to have an efficient means for
5 producing a series of structural analogues of these molecules in order to probe the mechanism of action of these molecules.

As shown in Figure 6, both CZ and Azinomycin B have α-acyloxy amine core structures. By labelling the
10 substituents off of the α-acyloxy amine core structure R_1, R_2 and R_3, a Passerini reaction scheme for the synthesis of Carzinophilin, Azinomycin B and their structural analogues is identified.

Once a Passerini reaction scheme for
15 Carzinophilin and Azinomycin B has been identified, a Passerini multicomponent combinatorial array synthesis of Carzinophilin and Azinomycin B analogues can be designed by varying the R_1, R_2 and R_3 substituents of the reaction components. One possible three dimensional
20 Passerini-type MCCA array synthesis of Carzinophilin and Azinomycin B structural analogues is provided in Figure 6.

A two dimensional MCCA array synthesis of Carzinophilin and Azinomycin B structural analogues
25 using three sets of diasteriometric isocyanates and two different aldehydes was performed. The combinatorial array synthesis and the yields obtained are provided in Table 2.

Table 2   Azinomycin Analogs Synthesized via Passerini Reactions

Cmpd	Isocyan	Aldehyde	Acid	Z/E	de[a]	% yield[b]	Major Product
109E	107E	(S)-2-methyl-glycidal	1-naphthoic acid	1/2.5	3.4:1	42%	
109Z	107Z	(S)-2-methyl-glycidal	1-naphthoic acid	3.9/1	3.4:1	53%	
110E	108E	(S)-2-methyl-glycidal	1-naphthoic acid	1/1.5	5:5:1:1	37%	
110Z	108Z	(S)-2-methyl-glycidal	1-naphthoic acid	3.3/1	3.4:3.4:1:1	51%	
111Z/E	106Z/E Z/E=1/1.4	(S)-2-methyl-glycidal	1-naphthoic acid	1.4/1	3:3:1:1	39%	
112Z/E	108Z/E Z/E=1/1.4	n-butyral	1-naphthoic acid	1/1	1:1	68%	
113Z/E	106Z/E Z/E=1/1.4	n-butyral	1-naphthoic acid	1/1.4	-----	69%	
114Z/E	107Z/E Z/E=1/1.4	n-butyral	1-naphthoic acid	1/1.2	-----	57%	

[a]Major product. [b]Combined Z/E isolated yields.

The isocyanates used in the combinatorial array synthesis described in Table 2 were prepared according to the general reaction scheme provided below.

5
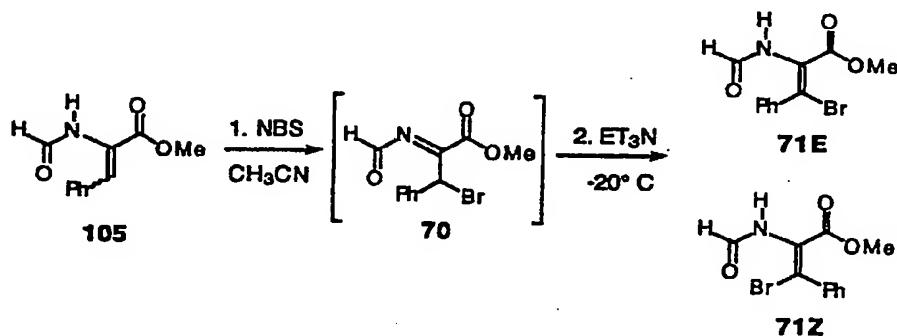
Methyl-isocyanoacetate and benzaldehyde were
10 reacted to form the dehydroaminoacid precursor 105 according to the procedure described in Hoppe, D., et al., U. Liebigs Ann. Chem. (1972) 766:116-129. The mixture of geometric isomers were then brominated with N-bromosuccinamide (NBS) in CH_3CN to yield the α-bromo
15 imine derivative 70 which tautermerized in Et_3N to afford the corresponding β-bromo-dehydroamino acids 71Z and 71E in 81% yield (Z/E = 1.4:1). The modified bromonation conditions (See Combs, A., et al., Tetrahedron Lett. (1992) 33:6419) gave substantially increased yields of
20 the E-isomer as compared to the conditions reported by Matsumoto. Nunami, K., et al., Tetrahedron (1988) 44:5467 (60% yield, Z/E = 13:1).

The resulting vinyl bromide diasteriomers
71E/Z were separated by silica gel chromatography and
25 recrystallized to analytical purity. Their stereochemistries were assigned according to the

35

literature by ^{1}H NMR chemical shift comparison of the
methyl proton resonances of the E- and Z- esters.

Isocyanides **106E** and **106Z** were formed from a
β-bromo-dehydroamino acids **71E** and **71Z** with retention of
5 the olefin configuration by dehydrating the N-formyl
groups with $POCl_3$ and Et_3N in CH_2Cl_2 at 0°C for 30
minutes.

The corresponding vinyl aziridine compounds
107E and **107Z** were formed from the isocyanates **106E** and
10 **106Z** by adding ethyleneimine to the isocyanates in
tetrahydrofuran and triethyl amine for 3-5 hours.

The reaction conditions and physical data for
isocyanates and the structural analogues produced in the
combinatorial synthesis described in Table 2 are
15 provided below.

The above-described combinatorial synthesis of
carzinophilin and azinomycin structural analogues
exposes the power of the present invention to enable the
synthesis of broad arrays of structural analogues. For
20 example, in the present synthesis of carzinophilin and
azinomycin structural analogues, it is possible to
further vary the aziridine ring, the phenyl ring and the
olefin portion of the isocyanate, the epoxide group of
the aldehyde as well as the substituents on the aromatic
25 ring of the acid. Thus, as can be seen from the present
example, a very broad array of carzinophilin and
azinomycin structural analogues may be produced
according to the present invention.

It may be further noted that the above-
30 described combinatorial array synthesis of carzinophilin
and azinomycin structural analogues may be readily
performed as a solid phase synthesis. For example, by
using a carboxylic acid containing a hydroxy, thio or
amino functional group, one could readily attach the
35 carboxylic acid to a solid support. Then, by adding an
aldehyde and an isocyanate under the conditions
described in the present example, one could carry out

the above-described synthesis as a solid phase
synthesis.

Experimental Data
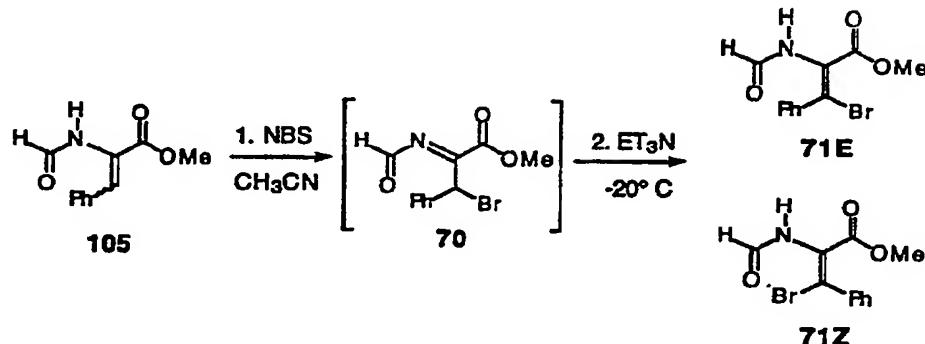
NBS (5.4 g, 30.22 mmol) was added to a r.t CH₃CN (20
5   mL) solution of dehydoamino acid **105** (5.9 g, 28.78
mmol). The reaction was stirred at 25°C for 1 hr. and
then heated to 45°C for 30 min. to give α-bromo imine
intermediate **70**. The solution of **70** was then cooled to
r.t. and Et₃N (4.0 mL, 31.7 mmol) added dropwise over a 5
10 min. period. This mixture was allowed to stir for 1
hr., after which the solvent was removed under reduced
pressure. The crude oil was taken up in EtOAc and the
insoluble succinimide removed by filtration. The
solvent was rotoevaporated to a clear oil, which was
15 further purified by flash silica gel chromatography to
yield two fractions: pure **71Z** (2.0 g) and bromides **71Z**
and **71E** (4.6 g, Z/E=1:1.2) for an 81% overall yield
(Z/E=1:1.4) The Z- and E- isomers could be further
separated by flash silica gel chromatography and then
20 recrystallized from EtOAc/Hex to analytical purity. The
E- and Z- isomer data was consistent with previous
literature assignments: **71E** m.p. 110-112°C, lit.m.p.
109-110°C; **71Z** m.p. 138-140°C, lit.m.p. 136-138°C.
NBS (34 mg, 0.188 mmol) was added to a r.t.
25 CD₃CN (0.5 mL) solution of dehydroamino acid **105** (35 mg,
0.171 mmol). After 3 hrs. the reaction gave the α-bromo
imine **70** quantitatively by ¹H and ¹³C NMR.

70: ^{1}H NMR (360 MHz, CD$_3$CN) δ 9.33 (s, 1H,
CHO), 7.53 (m, 2H, o-ArH), 7.39 (m, 3H, m,p-ArH), 6.26
(s, 1H, CHBr), 3.81 (s, 3H, OCH$_3$); ^{13}C NMR (90 MHz,
CD$_3$CN), δ 174.05 (CHO), 159.52 (C), 136.71 (C), 130.29
5   (CH), 130.25 (CH), 129.71 (CH), 54.86 (OCH$_3$), 48.86
(CHBr).
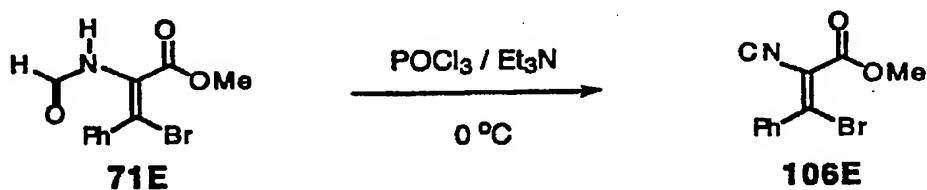
POCl$_3$ (217 µL, 2.3 mmol) was added dropwise over a
15 min. period to a 0°C CH$_2$Cl$_2$ (40 ml) solution of
bromide 71E (600 mg, 2.12 mmol) and Et$_3$N (1.4 mL, 10.6
10   mmol). The solution was allowed to stir at 0°C for 30
min., after which, the reaction was quenched with 20 mL
of aqueous Na$_2$CO$_3$ (450 mg, 4.24 mmol). This mixture was
stirred for 15 min. and then the aqueous layer extracted
3X with Et$_2$O. The combined organic layers were dried
15   over MgSO$_4$ and rotoevaporated to a brown oil. The crude
oil was triturated with Et$_2$O (3X) and the ether fractions
filter through a MgSO$_4$ plug. The ether was removed under
reduced pressure to afford 106E (590 mg, 105%) as a
brown oil. The extracted material was sufficiently pure
20   (>90%) for the next synthetic step without
chromatography. (The crude could be further purified by
flash silica gel chromatography, but with noticeable
loss in material).

106E: IR (film) cm^{-1}: 2956, 2112 (CN), 1744,
25   1699, 1442, 1261, 1119, 893; ^{1}H NMR (360 CHCl$_3$) δ 7.46
(m, 2H, o-ArH), 7.39 (m, 3H, m,p-ArH), 3.86 (s, 3H,
OCH$_3$); ^{13}C NMR (90 MHz, CHCl$_3$), δ 171.9 (CHO), 160.3 (C),
138.1 (C), 136.6 (C), 130.9 (CH), 128.5 (CH), 128.5

(CH), 128.5 (C), 53.4 (OCH$_3$). HRMS (CI) [MH]$^+$ calcd for C$_{11}$H$_8$NO$_2$Br 265.9817, found 265.9817.
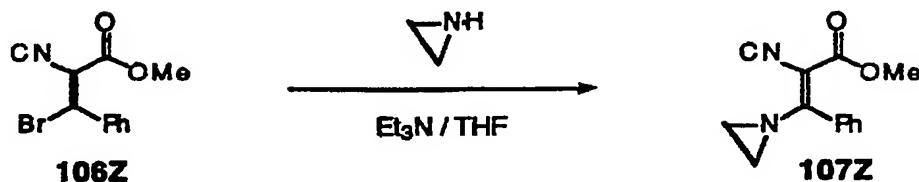
Ethyleneimine (125 μL, 2.43 mmol) was added to a r.t. THF (10 mL) solution of isocyanide bromide **106Z**
5 (215 mg, 0.81 mmol) and Et$_3$N (110 μL, 0.90 mmol). The mixture was stirred for 3 hrs., after which the solvent and excess amines were removed under reduced pressure to afford a brown oil. Attempts to extract over aqueous solutions hydrolyzed the vinyl aziridine. The crude oil
10 was therefore triturated with Et$_2$O and completely insoluble EtNH$^+$Br$^-$ removed by filtration through a MgSO$_4$ plug. The ether was rotoevaporated to a brown oil **107Z** (185 mg, 100%), which was sufficiently pure (>90%) for analytical data.
15   **107Z**: IR (film) cm^{-1}: 3350, 3000, 2952, 2116 (CN), 1560, 1290; ^{1}H NMR (360 MHz, CHCl$_3$) δ 7.41-7.26 (m, 3H, o,p-ArH), 7.16 (m, 2H, m-ArH), 3.59 (s, 3H, OCH$_3$), 2.34 (s, 4H, N(CH$_2$)$_2$); ^{13}C NMR (90 MHz, CHCl$_3$), δ 170.0 (C), 165.3 (C), 161.6 (C), 129.4 (CH), 128.0 (C), 127.9
20 (CH), 127.2 (CH), 51.9 (OCH$_3$), 29.9 (N(CH$_2$)$_2$); HRMS (CI) [MH]$^+$ calcd for C$_{13}$H$_{12}$N$_2$O$_2$ 229.0978, found 229.0977.

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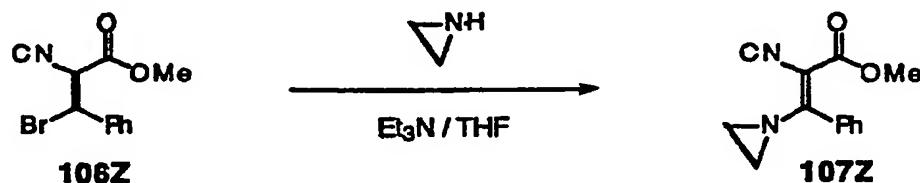
Ethyleneimine (145 µL, 2.89 mmol) was added to a
r.t. THF (5 mL) solution of isocyanide bromide **106E** (225
mg, 0.962 mmol) and Et$_3$N (150 µL, 1.16 mmol). The
mixture was stirred for 2 hrs. after which the solvent
5   and excess amines were removed under pressure to afford
a brown oil (attempts to extract over aqueous solutions
hydrolyzed the vinyl aziridine). The crude oil was
therefore triturated with Et$_2$O and the completely
insoluble EtNH$^+$Br$^-$ removed by filtration through a MgSO$_4$
10   plug. The ether was rotoevaporated to afford a brown
oil **107E** (255 mg, 103%), which was sufficiently pure
(<90%) for analytical data.

107E: IR (film) cm^{-1}: 3325, 3001, 2952, 2117
(CN), 1717, 1554, 1445, 1390, 1257, 1148, 1124; ^{1}H NMR
15   (360 MHz, CHCl$_3$) δ 7.40 (m, 5H, ArH), 3.81 (s, 3H, OCH$_3$),
2.25 (s, 4H, N(CH$_2$)$_2$); ^{13}C NMR (90 MHz, CHCl$_3$), δ 168.1
(C), 166.6 (C), 161.5 (C), 135.3 (C), 129.8 (CH), 128.3
(CH), 128.2 (C), 127.3 (CH), 52.1 (OCH$_3$), 32.9 (N(CH$_2$)$_2$);
HRMS (CI) [MH]$^+$ calcd for C$_{13}$H$_{12}$N$_2$O$_2$ 229.0977, found
20   229.0977.
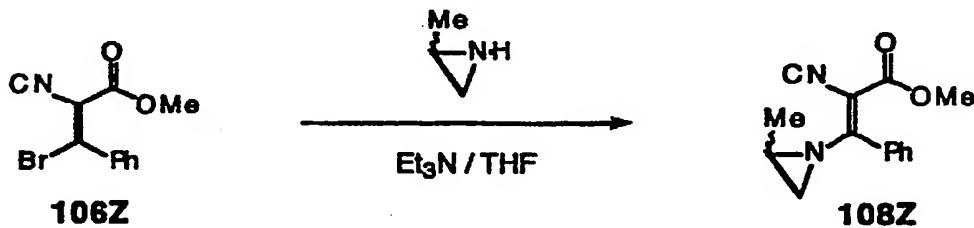
Racemic 2-methylasiridine (210 µL, 2.91 mmol)
was added to a r.t. THF (10 mL) solution of isocyanide
bromide **106Z** (0.88 mmol) and Et$_3$N (125 µL, 0.97 mmol).
The mixture was stirred for 2 hrs., after which the
25   solvent and excess amines were removed under reduced
pressure to afford a brown oil. The crude oil was
extracted 3X with Et$_2$O over H$_2$O. The ether fractions
were dried over MgSO$_4$ and rotoevaporated to afford a

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brown oil **108Z** (198 mg, 93%), which was sufficiently
pure (>90%) for analytical data.

   108Z: IR (film) cm^{-1}: 3350, 2953, 2116 (CN),
1723, 1564, 1291; ^{1}H NMR (360 MHz, CHCl$_3$) δ 7.44-7.35 (m,
5   3H, o,p-ArH), 7.21 (m, 2H, m-ArH), 3.61 (s, 3H, OCH$_3$),
2.54 (ddq, J=3.8, 5.5, 6.1 Hz, 1H, NCHCH$_3$), 2.23 (d,
1=6.1 Hz, 1H, NCH$_2$), 2.18 (d, J=3.8 Hz, 1H, NCH$_2$), 1.36
(d, J=5.5 Hz, 3H, NCHCH$_3$); ^{13}C NMR (90 MHz, CHCl$_3$), δ
169.9 (C), 165.8 (C), 161.8 (C), 135.0 (C), 129.4 (CH),
10   128.3 (C), 128.2 (CH), 127.4 (CH), 52.0 (OCH$_3$), 38.2
(NCHCH$_3$), 36.4 (NCH$_2$), 18.3 (NCHCH$_3$); HRMS (CI) [MH]$^+$
calcd for C$_{14}$H$_{14}$N$_2$O$_2$ 243.1134, found 243.1129.

106E   Et$_3$N / THF   **108E**
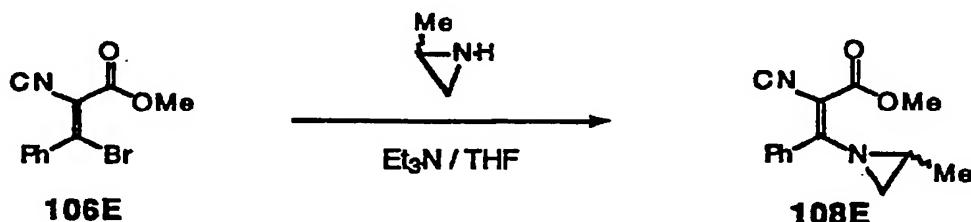
   Racemic 2-(methyl)-aziridine (250 µL, 3.0
mmol) was added to a r.t. THF (5 mL) solution of
15   isocyanide bromide **106E** (263 mg, 0.992 mmol) and Et$_3$N
(150 µL, 1.19 mmol). The mixture was stirred for 2
hrs., after which the solvent and excess amines were
removed under reduced pressure to afford a brown oil.
The crude oil was extracted 3X with Et$_2$O over H$_2$O to
20   efficiently remove the Et$_3$NH$^+$Br$^-$. The combined ether
fractions were dried over MgSO$_4$ and rotoevaporated to
afford a brown oil **108E** (269 mg, 112%), which was
sufficiently pure (>90%) for analytical data.

   108E: IR (film) cm^{-1}: 3303, 2995, 2952, 2117
25   (CN), 1723, 1548, 1445, 1172, 1124; ^{1}H NMR (360 MHz,
CHCl$_3$) δ 7.47-7.40 (m, 5H, ArH), 3.83 (s, 3H, OCH$_3$), 2.31
(ddq, J=1.5, 5.2, 6.1 Hz, 1H, NCHCH$_3$), 2.29 (d, J=1.5 Hz,
1H, NCH$_2$), 2.18 (d, J=5.2 Hz, 1H, NCH$_2$), 1.24 (d, J=6.1
Hz, 3H, NCHCH$_3$); ^{13}C NMR (90 MHz, CHCl$_3$), δ 167.8 (C),

166.7 (C), 161.7 (C), 135.8 (C), 129.8 (CH), 128.4 (C),
128.3 (C), 127.5 (CH), 52.1 (OCH$_3$), 40.2 (NCHCH$_3$) 39.9
(NCH2), 17.8 (NCHCH$_3$); HRMS (CI) [MH]$^+$ calcd for C$_{14}$H$_{14}$N$_2$O$_2$
243.1134, found 243.1134.
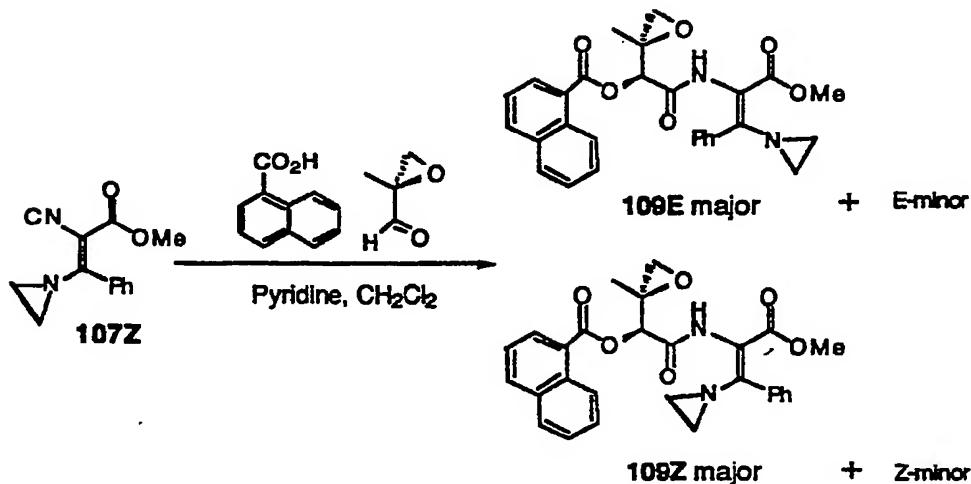
5   Pyridine (72 μL, 0.89 mmol), (S)-2-(methyl)-
glycidal (210 μL, 2.43 mmol), and 1-naphthoic acid (140
mg, 0.81 mmol) were added sequentially to a r.t. CH$_2$Cl$_2$
(5 mL) solution of isocyanide **107Z** (0.81 mmol). The
mixture was stirred under N$_2$ for 20 hrs., after which the
10   solvent was removed under reduced pressure. The crude
oil was immediately purified by flash silica gel
(deactivated with 5% Et$_3$N) chromatography (gradient
eluted with 100% hex to 1:1 hex/EtOAc) to afford four
fractions: **109Z** (138 mg, de=2.9:1), **109Z** (28.5 mg,
15   de=2:1), **109Z/109E** (13.0 mg, Z/E=2:1, de=1.4:1, de=1:2)
and **109E** (30 mg, de=2.3:1) for a 53% overall yield
(**109Z/109E=5.1:1**).

109Z (major diasteromer): IR (film) cm^{-1}: 3296, 3056, 2999, 2949, 1717, 1699, 1593, 1511, 1310, 1240, 1194, 1143, 1060; ^{1}H NMR (360 MHz, CDCl$_3$), δ 8.98 (d, l=8.8 Hz, 1H, ArH), 8.38 (dd, J=1.2, 7.3 Hz, 1H, ArH),

5   8.07 (d, J=8.1 Hz, 1H, ArH), 7.91 (d, J=7.6 Hz, 1H, ArH), 7.67-7.51 (m, 3H, ArH), 7.55 (bs 1H, NH), 7.37-7.25 (m, 5H, ArH), 5.39 (s, 1H, OCH), 3.49 (s, 3H, OCH$_3$), 3.14 (d, J=4.5 Hz, 1H, OCH$_2$), 2.86 (d, J=4.6 Hz, 1H, OCH$_2$), 2.09 (bs, 4H, N(CH$_2$)$_2$), 1.66 (s, 3H, OCCH$_3$); ^{13}C NMR

10   (90 MHz, CDCl$_3$) δ 166.87, 165.24, 164.07, 164.02, 136.73, 133.80, 133.56, 131.24, 130.42, 128.63, 128.41, 128.23, 127.87, 127.01, 126.24, 125.70, 125.55, 124.30, 108.27, 75.53, 55.78, 52.66, 51.52, 32.32, 17.49; HRMS (FAB) [MH]$^+$ calcd for C$_{28}$H$_{26}$N$_2$O$_6$ 487.1869, found 487.1869.
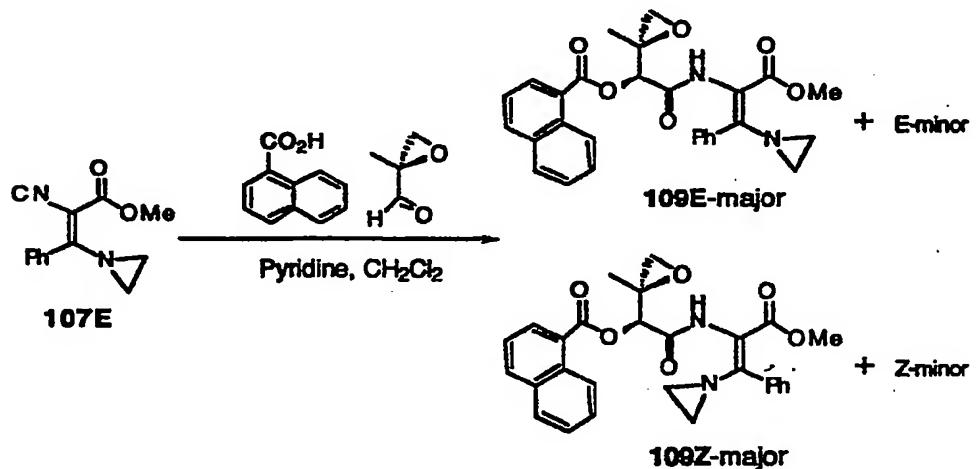
15   Pyridine (55 μL, 0.68 mmol), (S)-2-(methyl)-glycidal (155 μL, 1.78 mmol), and 1-naphthoic acid (101 mg, 0.59 mmol) were added sequentially to a r.t. CH$_2$Cl$_2$ (3.0 mL) solution of isocyanide **107E** (135 mg, 0.59 mmol). The mixture was stirred under N$_2$ for 27 hrs.,

20   after which the solvent was removed under reduced pressure. The crude oil was immediately purified by flash silica gel (deactivated with 5% Et$_3$N) chromatography (gradient eluted with 100% hex to 1:1

hex/EtOAc) affording two fractions: **109E** (84 mg, de=3.4:1) and **109Z** (30.5 mg, de=1.5:1), for a 42% overall yield (**109E/109Z=2.8:1**).

5 **109E** (major diasteromer): IR (film) cm^{-1}: 3311, 3058, 3001, 2948, 1722, 1511, 1279, 1244, 1194, 1138; ^{1}H NMR (360 MHz, CDCl$_3$), δ 8.85 (d, J=8.6 Hz, 1H, ArH), 8.10 (dd, J=1.1, 7.2 Hz, 1H, ArH), 8.02 (d, J=8.2 Hz, 1H, ArH), 7.86 (d, J=8.0 Hz, 1H, ArH), 7.62-7.43 (m, 3H, ArH), 7.28-7.15 (m, 5H, ArH), 6.99 (bs, 1H, NH), 5.12

10 (s, 1H, OCH), 3.76 (s, 3H, OCH$_3$), 2.82 (d, J=4.5 Hz, 1H, OCH$_2$), 2.63 (d, J=4.6 Hz, 1H, OCH$_2$), 2.21 (bs, 4H, N(CH$_2$)$_2$), 1.4 (s, 3H, OCCH$_3$); ^{13}C NMR (90 MHz, CDCl$_3$) δ 166.87, 165.24, 164.07, 164.02, 136.73, 133.80, 133.56, 131.24, 130.42, 128.63, 128.41, 128.23, 127.87, 127.01,

15 126.24, 125.70, 125.55, 124.30, 108.27, 75.53, 55.78, 52.66, 51.52, 32.32, 17.49; HRMS (FAB) [MH]$^+$ calcd for C$_{28}$H$_{26}$N$_2$O$_6$ 487.1869, found 487.1869.

Pyridine (36 μL, 0.45 mmol), (S)-2-(methyl)-glycidal (107 μL, 1.24 mmol), and 1-naphthoic acid (72

20 mg, 0.42 mmol) were added sequentially to a r.t. CH$_2$Cl$_2$ (5 mL) solution of isocyanide **108Z** (100 mg, 0.41 mmol). The mixture was stirred under N$_2$ for 24 hrs., after which the solvent was removed under reduced pressure. The

crude oil was immediately purified by flash silica gel (deactivated with 5% Et$_3$N) chromatography (gradient eluted with 100% hex to 1:1 hex/EtOAc) affording four fractions: **110Z** (69 mg, de=3.4:3.4:1:1), **110Z** (11 mg, de= 1.5:1.5:1:1), **110Z** and **110E** (14 mg, **110Z**, de=1.5:1.5:1:1 and **110E**, de=1:1) and **110E** (22 mg, de=1.5:1.5:1:1), for a 56% overall yield (**110Z/110E**=3.2:1).

110Z (major diasteromers): IR (film) cm^{-1}: 3290, 3064, 2994, 1715, 1511, 1328, 1237, 1138; 1H NMR (360 MHz, CDCl$_3$), δ 8.97 (d, J=8.7 Hz, 1H, ArH), 8.93 (d, J=9.0 Hz, 1H, ArH), 8.38 (dd, J=1.1, 7.3 Hz, 1H, ArH), 8.35 (dd, J=1.1, 7.4 Hz, 1H, ArH), 8.08 (d, J=8.3 Hz, 2H, ArH), 7.91 (d, J=7.9 Hz, 2H, ArH), 7.66-7.52 (m, 6H, ArH), 7.41 (bs, 1H, NH), 7.40 (bs, 1H, NH), 7.37-7.26 (m, 10H, ArH), 5.36 (s, 1H, OCH), 5.35 (s, 1H, OCH), 3.50 (s, 3H, CCH$_3$), 3.49 (s, 3H, OCH$_3$), 3.14 (d, J= 4.5 Hz, 1H, OCH$_2$), 3.12 (d, 4.6 Hz, 1H, OCH$_2$), 2.88 (d, J=1.3 Hz, 1H, OCH$_2$), 2.86 (d, J=4.0 Hz, 1H, OCH$_2$), 2.25 (m, 1H, NCHCH$_3$), 2.15 (m, 3H, NCHCH$_3$, NCH$_2$), 2.00 (d, J=6.1 Hz, 1H, NCH$_2$), 1.89 (d, J=3.7 Hz, 1H, NCH$_2$), 1.67 (s, 3H, OCCH$_3$), 1.66 (s, 3H, OCCH$_3$), 1.21 (d, J=6.1 Hz, 3H, NHCH$_3$), 1.09 (d, J=4.9 Hz, 3H, NHCH$_3$); ^{13}C NMR (90 MHz, CDCl$_3$) δ 166.26, 165.99, 165.87, 165.81, 164.28, 164.28, 158.57, 158.57, 136.29, 136.21, 134.10, 134.02, 133.79, 133.79, 131.42, 131.37, 130.82, 130.71, 128.58, 128.58, 128.41, 128.41, 128.31, 128.31, 128.08, 128.08, 127.64, 127.64, 126.40, 126.40, 125.98, 125.86, 125.65, 125.65, 124.50, 124.50, 111.06, 111.06, 76.23, 76.14, 56.29, 56.29, 53.61, 53.35, 51.44, 51.44, 37.54, 37.39, 36.40, 36.15, 18.32, 17.97, 17.48, 17.47; HRMS (DCI) [MH]$^+$ calcd for C$_{29}$H$_{28}$N$_2$O$_6$ 501.2026, found 501.2026.

108E

Pyridine, CH$_2$Cl$_2$

110E-major + E-minor

110Z-major + Z-minor
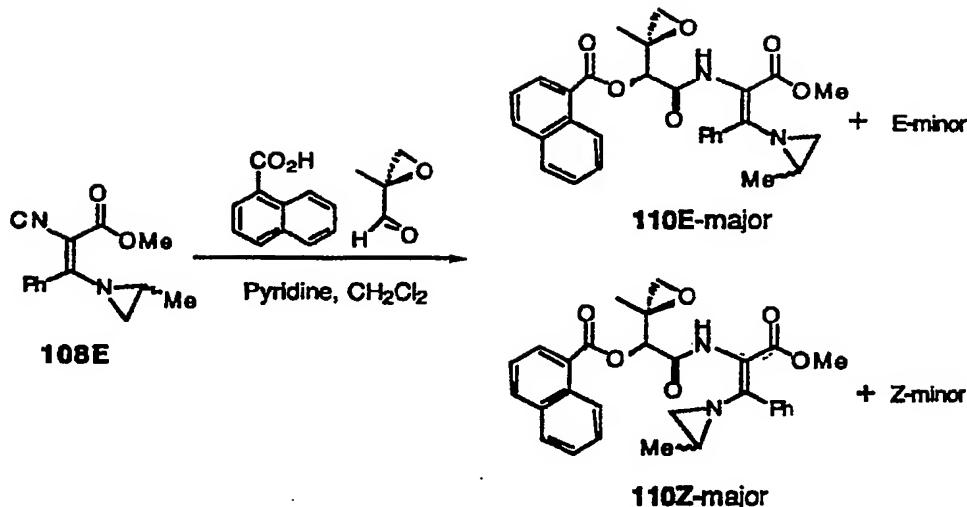
Puridine (40 μL, 0.50 mmol), (S)-2-(methyl)-glycidal (120 μL, 1.37 mmol), and 1-naphthoic acid (80 mg, 0.45 mmol) were added sequentially to a r.t. CH$_2$Cl$_2$ (3 mL) solution of isocyanide **108E** (110 mg, 0.45 mmol).

5   The mixture was stirred under N$_2$ for 24 hrs., after which the solvent was removed under reduced pressure. The crude oil was immediately purified by flash silica gel (deactivated with 5% Et$_3$N) chromatography (gradient eluted with 100% hex to 1:1 hex/EtOAc) affording four

10   fractions, **110Z** (20.5 mg, de=3:3:1:1), **110Z** and **110E** (23.8 mg, **110Z** de=2:2:1:1 and **110E**, de=1.5:1.5:1:1), **110E** (9.5 mg, de= 1:1), and **110E** (29 mg, de=2:2:1:1), for a 37% overall yield (**110E/110Z**=1.5:1).

   110E (major diasteromers): IR (film) cm^{-1}:

15   3322, 3055, 2995, 2946, 1711, 1505, 1275, 1239, 1136; ^{1}H NMR (360 MHz, CDCl$_3$), δ 8.86 (d, J=8.6 Hz, 1H, ArH), 8.82 (d, J=8.6 Hz, 1H, ArH), 8.14 (dd, J=1.2, 7.2 Hz, 1H, ArH), 8.05-8.01 (m, 3H, ArH), 7.87 (m, 3H, ArH), 7.65-7.40 (m, 5H, ArH), 7.3-7.2 (m, 10H, ArH), 6.94 (bs, 1H,

20   NH), 6.93 (bs, 1H, NH), 5.19 (s, 1H, OCH), 5.05 (s, 1H, OCH), 3.76 (s, 3H, OCH$_3$), 3.75 (s, 3H, OCH$_3$), 2.83 (d, J=5.0 Hz, 1H, OCH$_2$), 2.82 (d, J=5.7 Hz, 1H, OCH$_2$), 2.65 (d J=4.6 Hz, 1H, OCH$_2$), 2.63 (d, J=4.6 Hz, 1H, OCH$_2$), 2.30 (m, 1H, NCHCH$_3$), 2.25-2.10 (m, 3H, NCHCH$_3$, NCH$_2$),

25   2.15 (d, J=3.5 Hz, 1H, NCH$_2$), 2.14 (d, J=1.4 Hz, 1H,

NCH$_2$), 1.43 (s, 3H, OCCH$_3$), 1.40 (s, 3H, OCCH$_3$), 1.22 (d,
J=5.6 Hz, 3H, NHCH$_3$), 1.21 (d, J=5.5 Hz, 3H, NHCH$_3$); ^{13}C
NMR (90 MHz, CDCl$_3$) δ 167.07, 166.90, 165.43, 165.25,
164.17, 164.17, 164.14, 163.84, 137.10, 137.04, 133.88,
5 133.85, 133.69, 133.69, 131.35, 131.35, 130.54, 130.45,
128.66, 128.59, 128.49, 128.49, 128.24, 128.24, 127.97,
127.95, 127.20, 127.11, 126.34, 126.33, 125.91, 125.76,
125.67, 125.64, 124.41, 124.37, 107.72, 107.67, 75.72,
75.50, 55.92, 55.86, 52.95, 52.55, 51.59, 51.59, 39.91,
10 39.47, 39.32, 39.04, 18.03, 17.99, 17.82, 17.43; HRMS
(DCI) [MH]$^+$ calcd for C$_{29}$H$_{28}$N$_2$O$_6$ 501.2025, found 501.2026.
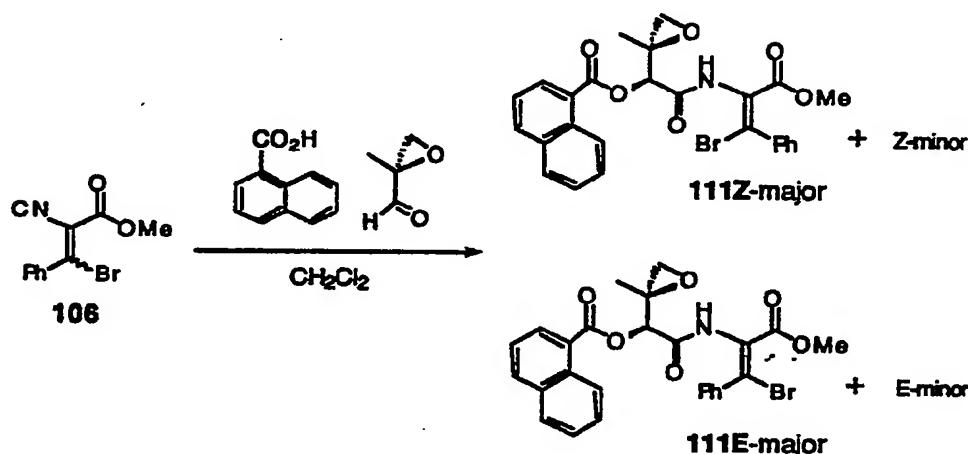
   (S)-2-(methyl)-glycidal (39 µL, 0.45 mmol) and
1-naphthoic acid (57.0 mg, 0.33 mmol) were added
sequentially to a r.t. CH$_2$Cl$_2$ (2 mL) solution of
15 isocyanide 106 (80.0 mg, 0.302 mmol, Z/E=1.4:1) and
stirred under N$_2$ for 24 hours. Additional (S)-2-
(methyl)-glycidal (20 µL, 0.23 mmol) was added and the
mixture stirred for 24 hours after which the solvent was
removed under reduced pressure. The crude oil was
20 purified by flash silica gel (deactivated with 5% Et$_3$N)
chromatography (gradient eluted with 100% hexane to 1:1
hex/EtOAc). One fraction was isolated containing an

inseparable mixture of all four stereoisomers **111** (61.0 mg, Z/E 1.4:1, de=3:3:1:1) for a 39% overall yield.

Major **111Z** and **111E**: IR (film) cm^{-1}: 3334, 2950, 1732, 1714, 1480, 1237, 1193, 1130, 783; ^{1}H NMR

5 (360 MHz, CDCl$_3$), δ 9.00-8.85 (m, 2H, ArH), 8.38 (m, 1H, ArH), 8.37-8.05 (m, 3H, ArH), 7.91 (s, 1H, NH), 7.89 (s, 1H, NH), 7.7-7.3 (m, 8H, ArH), 7.2-7.0 (m, 2H, ArH), 5.40 (s, 1H, OCH, Z-isomer), 5.24 (s, 1H, OCH, E-isomer), 3.92 (s, 3H, CO$_2$CH$_3$, E-isomer), 3.54 (s, 3H,

10 CO$_2$CH$_3$, Z-isomer), 3.13 (d, J=4.4 Hz, 1H, CCH$_2$, Z-isomer), 2.92 (d, J=4.3 Hz, 1H, CCH$_2$, E-isomer), 2.87 (d, J=4.4 Hz, 1H, OCH$_2$, Z-isomer), 2.71 (d, J=4.4 Hz, 1H, OCH$_2$, E-isomer), 1.63 (s, 3H, CCH$_3$, Z-isomer), 1.63 (s, 3H, CCH$_3$, E-isomer); ^{13}C NMR (90 MHz, CDCl$_3$) δ 165.30,

15 164.95, 164.17, 163.83, 162.82, 136.89, 135.99, 134.72, 134.39, 133.76, 131.46, 130.94, 130.72, 129.60, 129.52, 128.81, 128.61, 128.48, 128.40, 128.26, 127.79, 126.89, 126.59, 126.49, 126.45, 125.62, 125.49, 125.24, 124.45, 124.37, 120.13, 75.41, 75.21, 56.05, 55.76, 53.50,

20 52.76, 52.58, 17.86, 17.56, 17.37; HRMS (FAB) [MH]$^+$ calcd for C$_{26}$H$_{22}$NO$_6$Br 524.0709, found 524.0709.

108Z/E

Pyridine, CH$_2$Cl$_2$

112E

112Z
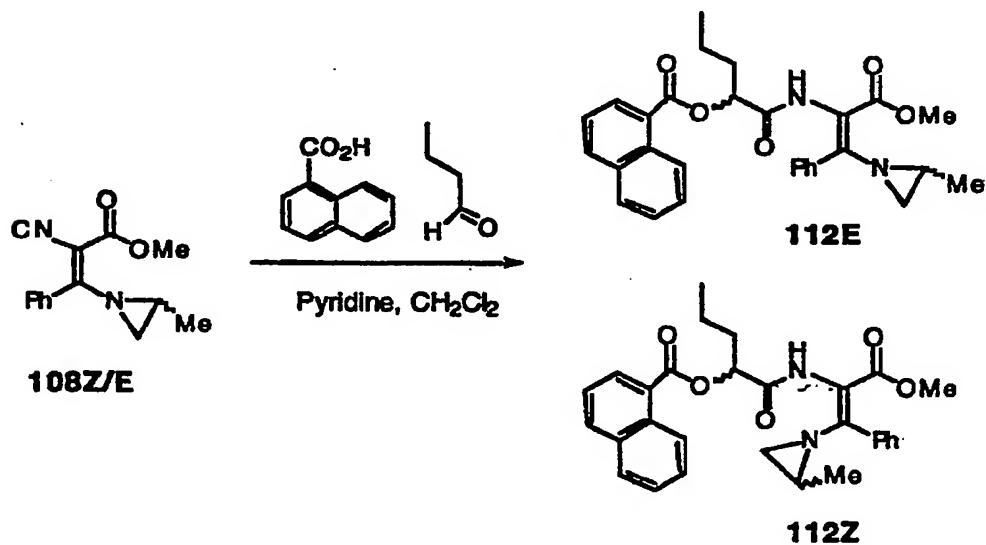
Pyridine (35 µL, 0.445 mmol), butyraldehyde (330 µL, 3.71 mmol), and 1-naphthoic acid (64 mg, 0.371 mmol) were added sequentially to a r.t. CH_2Cl_2 (3 mL) solution of isocyanide **108** (1:1.4 Z/E, 99.0 mg, 0.371

5 mmol). The mixture was stirred under N_2 for 22 hours, after which the solvent was removed under reduced pressure. The crude oil was immediately purified by flash silica gel (deactivated with 5% Et_3N) chromatography (gradient eluted with 100% hex to 1:1

10 hex/EtOAc) affording two fractions: **112Z** (61 mg, de=1:1, 34%) and **112E** (61 mg, de=1:1, 34%), for a 68% overall yield.

112Z: 1R (film) cm^{-1}: 3433.8, 3059, 2962, 1705, 1667, 1512, 1312, 1196, 1138; ^{1}H NMR (360 MHz, CDCl$_3$), δ

15 9.01 (d, J=8.7 Hz, 1H, ArH), 8.97 (d, J=8.5 Hz, 1H, ArH), 8.34 (dt, J=1.2, 7.5 Hz, 2H, ArH), 8.09 (d, J=8.2 Hz, 2H, ArH), 7.92 (d, J=8.1 Hz, 2H, ArH), 7.69-7.61 (m, 2H, ArH), 7.60-7.53 (m, 4H, ArH), 7.47 (bs, 1H, NH), 7.42 (bs, 1H, NH), 7.40-7.25 (m, 10H, ArH), 5.76 (t,

20 J=6.0 Hz, 1H, OCHCH$_2$), 5.72 (t, J=6.2 Hz, 1H, OCHCH$_2$), 3.48 (s, 3H, OCH$_3$), 3.47 (s, 3H, OCH$_3$), 2.20-2.11 (m, 7N, NCH$_2$, NCHCH$_2$, OCH$_2$CH$_2$), 2.03 (d, J=6.0 Hz, 1H, NCH$_2$), 1.95 (d, J=3.7 Hz, 1H, NCH$_2$), 1.85 (d, J=3.7 Hz, 1H, NCH$_2$), 1.71-1.60 (m, 4N, CHCH$_2$H$_2$), 1.09 (d, J=5.4 Hz, 6H,

25 NHCH$_3$), 1.05 (t, J=7.8 Hz, 6H, CH$_2$CH$_3$); ^{13}C NMR (90 MHz, CDCl$_3$) δ 169.36, 169.28 (CO$_2$CH$_3$), 165.99, 165.99 (C), 164.36, 164.36 (C), 158.08, 157.51 (C), 136.06, 135.00 (C), 134.19, 134.09 (CH), 133.82, 133.81 (C), 131.43, 131.37 (C), 130.40, 130.34 (CH), 128.64, 128.64 (CH),

30 128.39, 128.33 (CH), 128.15, 8.10 (CH), 127.59, 127.59 (CH), 126.44, 126.44 (CH), 125.99, 125.81 (C), 125.49 (CH), 124.41 (CH), 111.50, 111.46 (C), 74.37, 74.10 (CH), 51.44, 51.44 (OCH$_3$), 36.89, 36.74 (CH$_2$N), 36.59, 36.59 (CHN), 34.11, 34.04 (CH$_2$), 18.35, 18.23 (CH$_2$),

35 18.03, 17.92 (CHCH$_3$, 13.82, 13.82 (CH$_3$CH$_2$); HRMS (DCI) [MH]$^+$ calcd for $C_{29}H_{30}N_2O_5$ 487.2233, found 487.2233.

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112E: 1R (film) cm^{-1}: 3420, 3065, 2962, 1703, 1510, 1290, 1137; ^{1}H NMR (360 MHz, CDCl$_3$), δ 8.79 (d, J=8.0 Hz, 1H ArH), 8.70 (d, J=8.7 Hz, 1H, ArH), 8.04-8.01 (m, 2H, ArH), 7.93-7.87 (m, 4H, ArH), 7.65-7.50 (m,

5   4H, ArH), 7.48 (m, 2H, ArH), 7.30-7.10 (m, 10H, ArH), 6.90 (bs, 2H, NH), 5.42 (t, J=6.1 Hz, 1H, OCHCH$_2$), 5.41 (t, J=6.6 Hz, 1H, OCHCH$_2$), 3.74 (s, 6H, OCH$_3$), 2.32 (m, 1H, NCHCH$_3$), 2.26 (m, 1H, NHCH$_3$), 2.22 (d, J=7.4 Hz, 2H, NCH$_2$), 2.16 (d, J=1.8 Hz, 2H, NCH$_2$), 1.87 (m, 4H,

10   CH$_2$CH$_2$CH$_3$), 1.40 (m, 4H, CH$_2$CH$_2$CH$_3$), 1.24 (d, J=5.7 Hz, 3H, NCHCH$_3$), 1.21 (d, J=5.7 Hz, 3H, NCHCH$_3$), 0.92 (t, J=7.4 Hz, 6H, CH$_2$CH$_3$); ^{13}C NMR (90 MHz:, CDCl$_3$) δ 170.07, 169.96, 165.56, 165.54, 164.25, 164.25, 163.27, 162.99, 137.02, 137.02, 133.82, 133.76, 133.72, 133.69, 131.31,

15   131.24, 130.04, 129.92, 128.56, 128.51, 128.16, 128.14, 127.95, 127.93, 127.12, 127.09, 126.35, 126.35, 126.02, 125.97, 125.59, 125.55, 124.31, 124.29, 108.08, 108.02, 74.21, 74.16, 51.61, 51.61, 39.72, 39.72, 39.32, 39.29, 39.04, 39.04, 33.67, 33.67, 18.06, 18.02, 13.74, 13.74;

20   HRMS (DCI) [MH]$^+$ calcd for C$_{29}$H$_{30}$N$_2$O$_5$ 487.2233, found 487.2233.
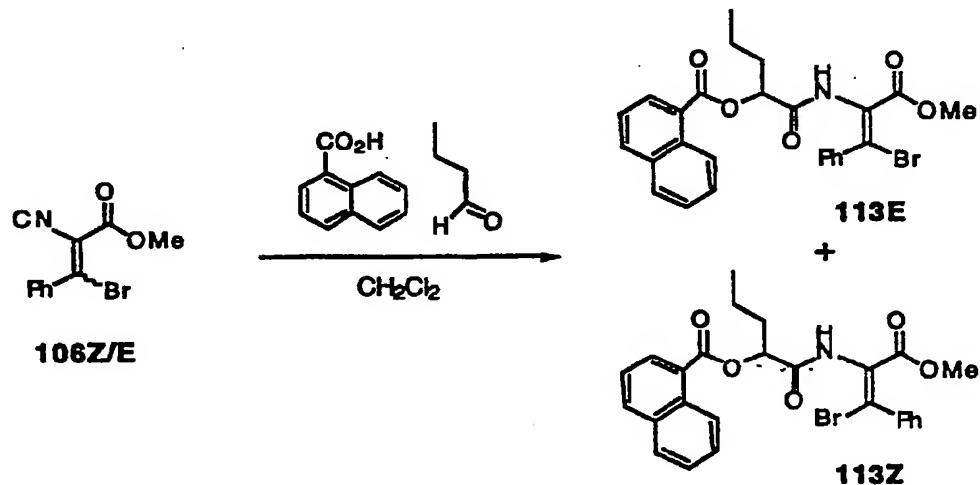
Butyraldehyde (3.65 μL, 4.04 mmol) and 1-naphthoic acid (80.0 mg, 0.45 mmol) were added sequentially to a r.t. CH_2Cl_2 (3 mL) solution of isocyanide **106** (98.0 mg, 0.405 mmol, E/Z=1.4:1) and

5   stirred under N_2 for 23 hours after which the solvent was removed under reduced pressure. The crude oil was purified by flash silica gel (deactivated with 5% Et_3H) chromatography (gradient eluted with 100% hex to 1:1 hex/EtOAc). One fraction was isolated as a white powder

10   containing an inseparable mixture of adducts **113** (140 mg, 69%, E/Z = 1.4:1).

113Z and **113E**: 1R (film) cm^{-1}: 3400, 3298, 3056, 2961, 2874, 1732, 1480, 1321, 1236, 1194, 1132, 783; ^1H NMR (360 MHz, $CDCl_3$), δ **113Z** 9.01 (d, J=8.6 Hz,

15   1H, **ArH**, Z-isomer), 8.36 (dd, J=1.2, 7.3 Hz, 1H, **ArH**, Z-isomer), 8.10 (d, J=8.3 Hz, 1H, **ArH**, Z-isomer), 7.91 (d, J=8.0 Hz, 2H, **ArH**, Z-isomer), 7.7-7.5 (m, 3H, **ArH**, Z-isomer), 7.4-7.2 (m, 3H, **ArH**, Z-isomer), 7.32 (bs, 1H, **NH**, Z-isomer), 7.05-6.85 (m, 3H, **ArH**), 5.69 (t, J=5.9

20   Hz, 1H, OCH_2CH_2, Z-isomer), 3.56 (s, 3H, CO_2CH_3, Z-isomer), 2.18-2.08 (m, 2H, OCH_2CH_2, Z-isomer), 2.01-1.92 (m, 2H, OCH_2CH_2, E-isomer), 1.68-1.56 (m, 2H, CH_2CH_3, Z-isomer), 1.03 (t, J=7.3 Hz, 3H, CH_2CH_3, Z-isomer), **113E** 8.87 (d, J=8.6 Hz, 1H, **ArH**, E-isomer), 8.12 (d, J=8.3

25   Hz, 1H, **ArH**, E-isomer), 8.05 (d, J=8.2 Hz, 1H, **ArH**, E-isomer), 7.76 (dd, J=1.2, 7.2 Hz, 1H, **ArH**, E-isomer), 7.7-7.5 (m, 3H, **ArH**, E-isomer), 7.4-7.2 (m, 3H, **ArH**, E-isomer), 7.32 (bs, 1H, **NH**, E-isomer), 5.50 (t, J=6.0 Hz, 1H, OCH_2CH_2, E-isomer), 3.94 (s, 3H, CO_2CH_3, E-isomer),

30   1.52-1.40 (m, 2H, CH_2CH_3, E-isomer), 0.954 (t, J=7.4 Hz, 3H, CH_2CH_3, E-isomer); ^{13}C NMR (90 MHz, $CDCl_3$) δ 168.10 (C), 167.18 (C), 165.58 (C), 165.06 (C), 163.92 (C), 162.89 (C), 136.71 (C), 135.77 (C), 134.43 (CH), 134.27 (CH), 133.85 (C), 133.75 (C), 131.46 (C), 131.41 (CH),

35   130.50 (CH), 130.15 (CH), 129.46 (CH), 129.42 (CH), 128.78 (CH), 128.74 (CH), 128.65 (CH), 128.59 (CH), 128.40 (CH), 128.40 (CH), 128.26 (CH), 128.23 (CH),

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127.86 (C), 127.23 (C), 126.49 (CH), 126.49 (CH), 125.57
(CH), 125.44 (CH), 125.38 (C), 124.86 (C) 124.38 (CH),
124.27 (CH), 118.98 (C), 114.48 (C), 73.78 (CH), 73.41
(CH), 52.72 (CH3), 52.57 (CH$_3$), 33.78 (CH$_2$), 33.60 (CH$_2$),
5   18.06 (CH$_2$), 17.98 (CH$_2$), 13.76 (CH$_3$), 13.67 (CH$_3$); HRMS
(FAB) [MH]$^+$ calcd for C$_{26}$H$_{24}$O$_5$NBr 510.0916, found 510.0916.
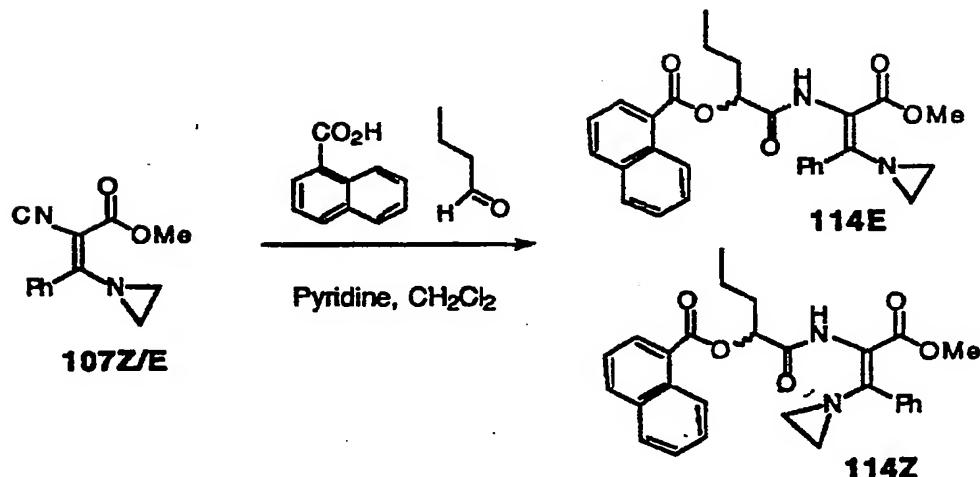
Pyridine (35 μL, 0.441 mmol), butyraldehyde
(330 μL, 3.67 mmol), and 1-naphthoic acid (63 mg, 0.367
mmol) were added sequentially to a r.t CH$_2$CL$_2$ (3 mL)
10   solution of isocyanide 107 (90.0 mg, 0.367 mmol,
Z/E=1:1.4). The mixture was stirred under N$_2$ for 12
hours after which the solvent was removed under reduced
pressure. The crude oil was immediately purified by
flash silica gel (deactivated with 5% Et$_3$N)
15   chromatography (gradient eluted with 100% hex to 1:1
hex/EtOAc) affording 2 fractions: 114Z (54.5 mg, 31%)
and 114E (44.5 mg, 26%), for a 57% overall yield
(Z/E=1.2:1).

114Z: IR (film) cm^{-1}: 3281, 3002, 2964, 1723, 1697, 1414, 1198, 1138; ^{1}H NMR (360 MHz, CDCl$_3$), δ 9.01 (d, J=8.3 Hz, 1H, ArH), 8.34 (dd, J=1.2, 7.2 Hz, 1H ArH), 8.10 (d, J=8.1 Hz, 1H, ArH), 7.92 (d, J=7.7 Hz, 1H
5   ArH), 7.66 (dt, J=1.4, 5.8 Hz, 1H ArH), 7.60-7.54 (m, 2H, ArH), 7.43 (bs, 1H, NH), 7.35-7.33 (m, 3H, ArH), 7.28-7.29 (m, 2H, ArH), 5.69 (t, J=6.12 Hz, 1H, OCHCH$_2$), 3.47 (s, 3H, OCH$_3$), 2.19-2.13 (m, 2H, OCHCH$_2$), 2.04-1.97 (AB, 4H, (CH$_2$)$_2$N), 1.7-1.6 (m, 2H, CH$_2$CH$_3$), 1.05 (t, J=7.3
10   Hz, 3H, CH$_2$CH$_3$); ^{13}C NMR (90 MHz, CDCl$_3$) δ 169.24 (C), 166.11 (C), 164.38 (C), 157.49, 136.06, 134.29, 133.91, 131.51, 130.56, 128.72, 129.49, 128.34 (CH), 128.25, 127.75 (CH), 126.50 (C), 125.78 (C), 125.55 (C), 124.50 (C), 112.01 (C), 74.46 (OCHCH$_2$), 51.56 (OCH$_3$), 34.06
15   (CHCH$_2$), 29.64 (N(CH$_2$)$_2$), 18.42 (CH$_2$CH$_3$), 13.85 (CH$_2$CH$_3$); HRMS (DCI) [MH]$^+$ calcd for C$_{28}$H$_{28}$N$_2$O$_5$ 473.2077, found 473.2070.

114E: IR (film) cm^{-1}: 3291, 2960, 2874, 1713, 1683, 1505, 1279, 1246, 1195, 1135, 784; ^{1}H NMR (360 MHz,
20   CDCl$_3$), δ 8.75 (d, J=8.4 Hz, 1H, ArH), 8.03 (d, J=8.1 Hz, 1H, ArH), 7.92 (dd, J=1.2, 7.4 Hz, 1H, ArH), 7.89 (dd, J=1.4, 7.8 Hz, 1H, ArH), 7.59 (AB, J=1.6, 6.9 Hz, 1H, ArH), 7.55 (AB, J=1.3, 6.9 Hz, 1H, ArH), 7.44 (dd, J=7.3, 8.2, 1H, ArH), 7.27-7.18 (m, 4H, ArH), 7.17-7.10
25   (m, 1H, ArH), 6.92 (bs, 1H, NH), 5.41 (t, J=6.2 Hz, 1H, OCHCH$_2$), 3.75 (s, 3H, OCH$_3$), 2.24-2.21 (m, 4H, (CH$_2$)$_2$N), 1.90 (m, 2H, OCHCH$_2$), 1.42-1.35 (m, 2H, CH$_2$CH$_3$), 0.917 (t, J=7.3 Hz, 3H, CH$_2$CH$_3$); ^{13}C NMR (90 MHz, CDCl$_3$) δ 170.00, 165.56, 164.24, 163.28, 136.80, 133.84, 133.74,
30   131.31, 130.03, 128.68, 128.53, 128.26, 127.99, 127.06, 126.39, 125.98, 125.60, 124.32, 108.71, 74.19, 51.67, 33.67, 32.33 ((CH$_2$)$_2$N), 18.06, 13.75; HRMS (DCI) [MH]$^+$ calcd for C$_{28}$H$_{28}$N$_2$O$_5$ 473.2077, found 473.2076.

II
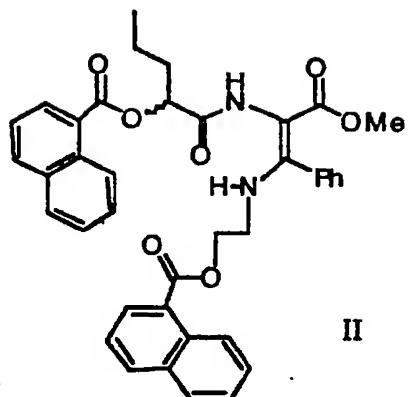
The nucleophilic addition of 1-naphthoic acid to the vinyl aziridine isocyanides is a common side reaction observed when excess 1-naphthoic acid or no pyridine was added to these Passerini reactions.

5   **II** : 1R (film) cm^{-1}: 3281, 3057, 2959, 1717, 1663, 1591, 1515, 1280, 1241, 1138; ^{1}H NMR (360 MHz, CDCl$_3$), δ 9.31 (t, J=5.8 Hz, 1H, NHCH$_2$), 8.85 (d, J=9.0 Hz, 1H, ArH), 8.77 (d, J=8.3 Hz, 1H, ArH), 8.25 (d, J=8.4 Hz, 1H, ArH), 8.04 (t, J=8.0 Hz, 2H, ArH), 7.98

10 (d, J=7.3 Hz, 1H, ArH), 7.89 (t, J=9.1 Hz, 2H, ArH), 7.63-7.46 (m, 6H, ArH), 7.34-7.22 (m, 5H, ArH), 6.62 (bs, 1H, NHCO), 5.29 (t, J=6.2 Hz, 1H, CHO), 4.33 (t, J=5.4 Hz, 2H, CH$_2$O), 3.66 (s, 3H, OCH$_3$), 3.34 (q, J=5.8 Hz, 2H, CH$_2$NH), 1.7-1.6 (m, 2H, CH$_2$CH), 1.3-1.2 (m, 2H,

15 CH$_2$CH$_3$), 0.84 (t, J=7.2 Hz, 3H, CH$_3$CH$_2$); LRMS (CI$^+$) [MH]$^+$ calcd for C$_{39}$H$_{36}$N$_2$O$_7$, 645.25, found 645.0.

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EXAMPLE 4 Structural Activity Profile of the *In vitro*
          Cytotoxicities Of The Carzinophilin and
          Azinomycin Analogs Of Example 3

          A structural activity relationship (SAR) of

5 the *in vitro* cytotoxicities of the Carzinophilin and
   Azinomycin analogs of Example 3 is provided in Table 3
   below.

Table   3 *In Vitro* Cytotoxicities of **Azinomycin Analogs** in HCT116
           Human Colon Carcinoma Cell Lines

	IC$_{50}$ (μM)		
Cmpd No.	HCT116	HCT116/VM46	HCT116/VP35
109E	4.39	5.56	5.27
109Z	5.4	1.6	2.6
110E	12.4	13.2	11.0
110Z	6.76	7.7	6.4
111E/ 111Z	>30	>30	>30
112E	>30	>30	>30
112Z	25.3	27.2	25.5
113E/ 113Z	>30	>30	>30
114E	>30	>30	>30
114Z	28.6	38.4	27.3
azinomycin B	0.838	—	—

[1]Cytotoxicity assessed by XTT assay after 72 hours continuous drug exposure.

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Each analog was incubated for 72 hours with
the human carcinoma cell line HCT116 and two drug
resistant sublines HCT116/VM46 and HCT116/VP35.
HCT116/VM46 was selected for resistance to VM-26 and
5   expresses the multidrug resistant (MDR) phenotype.
HCT116/VP35 was selected for resistance to VP-16 and is
resistant to topoisomerase II active drugs. The results
of the

Analogs **109E**, **109Z**, **110E** and **110Z** which all
10 contain vinyl-aziridine, epoxide and naphthoate moieties
displayed IC_{50}'s well within an order of magnitude of the
natural product, Azinomycin B. Interestingly, the
racemic 2-(methyl)-aziridine analogs **110E** and **110Z**
behaved nearly identically in the *in vitro* assays to the
15 correspondingly unsubstituted aziridine analogs **109E** and
109Z.

Analogs lacking the vinyl-aziridine moiety
gave no measurable cytotoxicity ($IC_{50}<30\mu M$) in all cases
studied. Substitution of the epoxide fragment for the
20 n-butyl group in analogs **114E**, **114Z**, **115E** and **115Z**
displayed significantly less activity when compared to
the most active compounds which contain the epoxide
moiety.

In addition, these compounds did not show
25 decreased potency when tested with Multiple Drug
Resistance (MDR) and topoisomerase II resistant cell
lines. This suggests that these compounds are not
substrates for the P-glycoprotein efflux pump nor do
they interact with topoisomerase II.

30 EXAMPLE 5 Synthesis of Peptidomimetic Polymer MCCA
            Array Using The Ugi Reaction

A combinatorial array of peptidomimetic
polymers having a non-hydrolyzable ethylenediamine core
structure can be prepared using the Ugi reaction. As
35 depicted in Figure 7, the Ugi reaction is a four
component condensation involving a first amino acid, a

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second amino acid having a Boc-protected alpha amino group, methylisocyanide and acetic acid. The Ugi reaction is conducted at room temperature in aqueous solvents. As is illustrated in Figure 7, once the
5   protecting groups have been removed, the Ugi reaction provides nonhydrolizable peptide analogues that mimic the structure of natural peptides.

EXAMPLE 6 Synthesis Of A Phosphotyrosine Peptide
10   Structural Analog MCCA Array
                     Using The Ugi Reaction.

         One application for an Ugi MCCA array synthesis is the preparation of phosphotyrosine peptide structural analogues for use as structure-based
15   competitive inhibitors. As depicted in Figure 8, phosphotyrosine peptides have a peptide backbone core structure. Structural variants of phosphotyrosine can be synthesized by varying the first and second amino acids employed (aa1 and aa2) as well as the other
20   components of the Ugi reaction.
         Several different combinatorial arrays of phosphotyrosine peptide analogues can be produced using the Ugi reaction. For example, as depicted in Figure 9a, pseudosubstrate peptide inhibitors can be prepared.
25   The amino acid side chains of the tyrosine peptide analogues are determined by which R_1 and R_2 groups are used for the isocyanide and the acid. Branched chain tyrosine-based peptide analogues, not otherwise accessible using standard solid phase synthesis, can be
30   prepared when R_3 on the amine is an amino acid or derivative thereof.
         As depicted in Figure 9b, low molecular weight analogues of tyrosine that possess an intact tyrosine structural unit can be prepared wherein the R groups of
35   the isocyanides, acids and amines employed are alkyl, aryl or acyl groups.

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As depicted in Figure 9c, low molecular weight derivatives of tyrosine where the tyrosine structural unit is also modified can be prepared by using different aldehydes.

As depicted in Figure 9d, low molecular weight tyrosine derivatives can be prepared where the acid and amine are the same molecule. Further variations of these tyrosine-based peptide analogues can also be made according to the present invention by using different reaction components.

EXAMPLE 7 Synthesis of Solid Support And Linker
        System For Use In Solid Phase Synthesis Of
        MCCA Array.

A proposed synthesis of a novel support and linker system is depicted in Figure 10. The linker can be synthesized by condensing ethylene oxide with the lithium enolate of succinic anhydride. The resulting alcohol can then be converted to its mesylate and displaced with sodium azide to provide the azide functionality. This anhydride is then heated in the presence of the polymer in dimethylformamide to afford a free carboxylate linker.

EXAMPLE 8 Synthesis of Passerini-type MCCA
        Array Using Solid Phase Synthesis

A solid phase MCCA array synthesis utilizing the Passerini reaction can be conducted using the linker described in Example 7. The carboxylic acid linker-resin system formed in Example 7 can be treated with dicyclohexylcarbodiimide and hydroxybenzotriazole in the presence of the methyl ester of alanine. The progress of the reaction can be monitored by nuclear magnetic resonance based on the disappearance of free alanine. Filtration of reactants and subsequent washing with fresh DMF should provide a purified resin-linker-amino

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acid system. Hydrolysis of the ester of alanine under
base conditions should then provide the free carboxylic
acid. Again, washing the resin with water provides a
resin-linker-alanine carboxylic acid free of impurities

5   or reactant contamination. Addition of cinnamaldehyde
and 2,2-diethylphosphonomethylisocyanide to the resin
reaction chamber stirring in an ethyl acetate solution
should result in the three component Passerini
condensation reaction. These reactants can be removed

10   after the reaction by washing. Addition of triphenyl
phosphine to a DMF solution of resin followed by washing
with solvent should provide an amine side-chain on the
linker. Heating this material should then cause an
intramolecular reaction wherein the linker forms a

15   lactam and releases the amine into solution.

       EXAMPLE 9 Synthesis Of Peptidomimetics Using
                Solid Phase Synthesis

            The use of the Ugi reaction as a means for
synthesizing peptide mimics is described in Example 5

20   and 6 and as depicted in Figure 11. By coupling the
first amino acid (aa_1) to a solid phase support as
described in Example 8, the solid state synthesis of
these peptide mimics can be performed. Reaction of the
linked amino acid with an isocyanide, an aldehyde and an

25   amine should yield a solid phase linked peptidomimetic
analog. Removal of the tBoc blocking group under Ugi
reaction conditions set forth in Figure 8 should afford
a product containing two amino acid components. This
process can be repeated over several cycles to provide 9^n

30   products where n is the number of cycles. Thus, this
application of the Ugi reaction provides numerous
analogues despite the use of a very small number of
different components.

EXAMPLE 10   Isolation Of Products From Ugi-type MCCA
                    Array Synthesis By Sequestering
                    The Unreacted Components

          The reaction products of an Ugi-type reaction
5 can be efficiently recovered from the reaction media by
sequestering the unreacted components thereby yielding a
substantially pure product.

          In an Ugi-type reaction, the unreacted
components consist of an aldehyde, an amine, an acid and
10 an isocyanide. A mixed bed resin containing strongly
acidic and basic resins can be used to remove the amine
and acid respectively. In order to isolate the product
of an Ugi reaction, the solvent is first removed by
evaporation at reduced pressure. Distilled water is
15 then added to the reaction mixture. To the resulting
aqueous solution is added a mixed bed resin containing
Biorad AG 501-X8/Bio-Rex MSZ 501, a strongly basic anion
exchange and strongly acidic cation exchange resin.
Once the mixed bed resin has been added, the solution is
20 stirred for 10 min. The mixture is then filtered and
concentrated to yield a substantially pure Ugi-type
reaction product.

          The use of exchange resins to sequester
unreacted acids or amines can be extended to other
25 unreacted components having different functional groups,
such as esters, where the unreacted component is
selectively converted to either an acid or amine after
the reaction, thereby enabling its removal by the resin.
          Metal sequestering agents can also be used
30 where organometallic solution chemistry is employed in
the MCCA reaction. For example, the addition of an EDTA
molecule tethered to a polymer can be used to sequester
palladium salts from the reaction mixture. Strongly
acidic cation exchange resins can be used to remove
35 boronic acids.

Example 11   Synthesis And Cleavage Of Passerini
                  Reaction Products From Photocleavable
                  <u>Carbamate Linker</u>

              Synthesis of photocleavable carbamate linkers
5 is depicted below. The photocleavable carbamate linkers
   120 and **121** were constructed by reacting isocyanates **123**
   and **124** with the hydroxyl group from the linker alcohol
   described in Williams, P., et al., <u>Tetrahedron</u> (1991)
   <u>47</u>:9867-9880.

10   The carbamate linkers **120** and **121** were then
   coupled to methylbenzhydrylamine (MBHA) Gly-resin **125**
   using HOBt in DMF to yield the polymer supported
   photocleavable carbamates **126** and **127**, respectively.
   Hydrolysis of the ester of carbamate **126** with LiOH,
15 THF/H$_2$O yielded the corresponding polymer supported acid
   128 in less than one hour. Surprisingly, the benzoate
   ester of carbamate **127** was resistant to hydrolysis under
   a variety of reaction conditions (LiOH, THF/H$_2$O, K$_2$CO$_3$,
   MeOH, NaOH, THF/H$_2$O and NaOH, MeOH).

20   Using the polymer supported acid **128**, a solid
   phase Passerini reaction was conducted. A CH$_2$Cl$_2$
   solution containing methyl isocyanoacetate and
   butyraldehyde were added to the polymer supported acid
   128 to yield the resin bound Passerini adduct **129**.

25   Initially, carbamate cleavage was attempted in
   methanol which yielded only the α-acyloxy hydrolysis
   Passerini product **131**. In order to avoid hydrolysis and
   capture the free amine product, photolysis at 350nm in a
   rayonet for 24 hours was conducted in CH$_3$CN in the
30 presence of acetic anhydride (10 equiv.). Excess
   solvent acylating agent were then removed under vacuum
   to yield **134** as the only product. No other products
   were detectable from the crude product by ^{1}H NMR and TLC.

Experimental Data
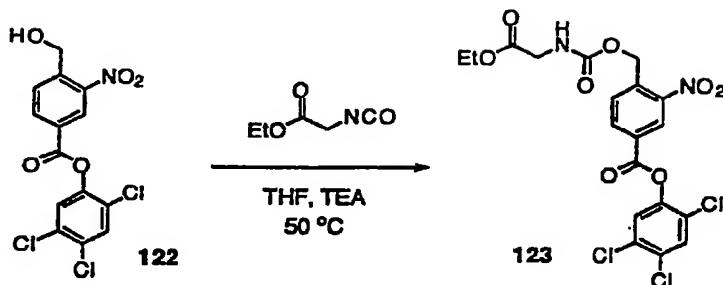
Ethyl-isocyanatoacetate (155 μL, 1.37 mmol) was added to
a THF (5 mL) solution of linker **122** (450 mg, 1.14 mmol).
Triethylamine was then added and the mixture allowed to
5   stir at 25°C overnight. TLC showed that the reaction
was slow, so the reaction was heated to 50°C. TLC
showed that the reaction was complete in 2 days. Upon
cooling the flask to -20°C, a precipitate formed and was
subsequently filtered off. ^{1}H NMR in CD_3CN showed that
10 this compound was the isocyanate decomposition product,
glycine ethyl ester. The filtrate solvent was then
removed under reduced pressure and the crude oil was
extracted 3X with CH_2Cl_2 over KH_2PO_4 buffer. The combined
organic layers were dried over Na_2SO_4 and evaporation of
15 the solvent under reduced pressure gave a yellow solid.
Purification by flash silica gell chromotography
(gradient eluted 100% hex to 1:1 hex/EtOAc) afforded
carbamate **123** (373 mg, 64%) as a white solid (mp 131-
133°C).
20   **123**: IR (film) cm^{-1}: 3323, 3097, 2985, 1750,
1699, 1538, 1461, 1351, 1248, 1206, 1084, 1060; ^{1}H NMR
(360 MHz, $CDCl_3$), δ 8.88 (d, J=1.7 Hz, 1H, ArH), 8.42
(dd, J=1.7, 8.2 Hz, 1H, ArH), 7.82 (d, J=8.2 Hz, 1H,
ArH), 7.60 (s, 1H, ArH), 7.44 (s, 1H, ArH), 5.62 (s, 2H,
25 OCH_2Ar), 5.61 (bt, J=5.3 Hz, 1H, $HNCH_2$), 4.22 (q, J=7.2
Hz, 2H, OCH_2CH_3), 3.99 (d, J=5.6 Hz, 2H, $HNCH_2$), 1.28 (t,
J=7.3 Hz, 3H, CH_2CH_3); ^{13}C NMR (90 MHz, $CDCl_3$) δ 169.75,

161.44, 155.43, 147.07, 145.38, 139.34, 134.88, 131.63, 131.12, 131.08, 128.87, 128.65, 126.74, 126.00, 124.14, 63.28, 61.61, 42.75, 14.05; HRMS (CI$^+$ [MH]$^+$ calcd for C$_{19}$N$_{15}$N$_2$O$_8$Cl$_3$ 504.9972, found 504.9972.
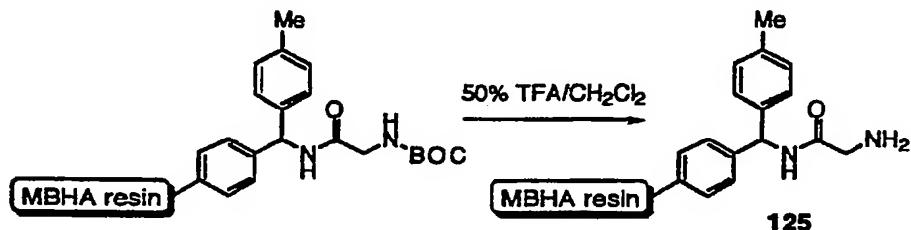
5   BOC-Gly-resin was deprotected with 33% TFA in CH$_2$Cl$_2$ for 30 min. and then washed exhaustively with CH$_2$Cl$_2$. The resin was then free baeed with 5% Hunig's base in CH$_2$Cl$_2$ and the solution filtered after 2 minutes. The resin was then washed exhaustively with sequential

10   applications of CH$_2$Cl$_2$/MeOH/CH$_2$Cl$_2$ until the resin appeared colorless. The resin was dried *in vacuo* overnight. The entire procedure was repeated again with 50% TFA in methylene chloride (20 mL) affording Gly-MBHA-resin **125** (1.39 g) as colorless beads. The

15   qualitative ninhydrin test (Kaiser Test) was positive (dark blue).
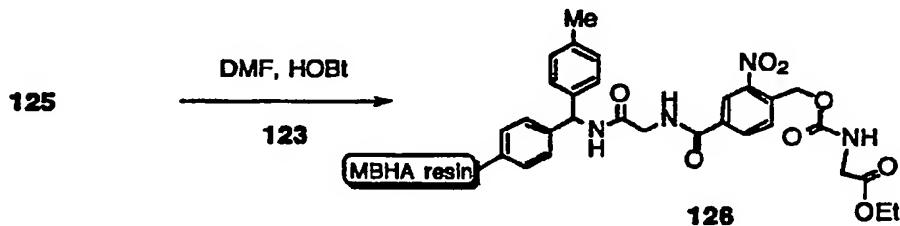
       Gly-carbamate **123** (1.2 g, 2.37 mmol) in 2 mL DMF was added to a 5 mL DMF suspension of MBHA-resin **125**

(1.34 g, 1.12 equiv./gram, obtained from Novabiochem.)
HOBt (470 mg, 3.4 mmol) was then added and the mixture
allowed to stir at 25°C for 36 hours. The resin was
washed exhaustively in sequential applications of
5   CH_2Cl_2/MeOH/CH_2Cl_2. After two iterations of this process,
resin **126** (1.83 g) was afforded as pale yellow beads.
The qualitative ninhydrin test (Kaiser Test) was
negative.

         Capping of residual free amines on the dried
10 resin **126** (1.7 g, ≈1.7 mmol) was performed by suspending
the resin in a pyridine (10 mL) solution of Ac_2O (1.7 mL,
16.9 mmol) and a catalytic amount of DMAP (25 mg, 0.2
mmol). The solution was stirred at 25°C for 1 hr. and
then filtered and washed exhaustively with sequential
15 applications of CH_2Cl_2/MeOH/CH_2Cl_2. The resin was dried
in vacuo overnight affording resin **126** (1.7 g) as pale
yellow beads. The qualitative ninhydrin test (Kaiser
Test) was negative.

126 → 1N LiOH / THF/H2O → **128**
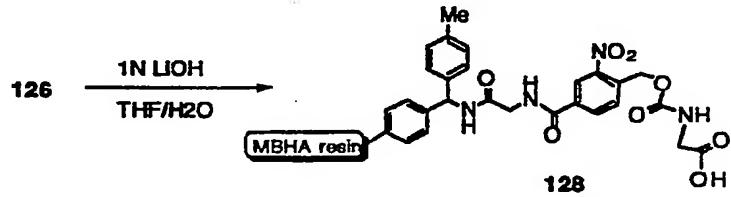
         (Ethyl)-glycinate-MBHA-Gly-resin **126** (1.6 g, ≈ 1.5
20 mmol) was stirred in 45 mL THF with 1N LiOH (15 mL, 15.0
mmol) for 1 hr. The mixture was then acidified with 1N
KH_2SO_4 (≈17 mL) to pH=2-3 and the resin was filtered.
Exhaustive sequential washings with H_2O/THF/CH_2Cl_2 and
subsequent drying *in vacuo* overnight afforded (glycinic
25 acid)-Gly-MBHA resin **128** (1.46 g) as pale yellow beads.
Photolysis of this resin in CH_3N and AC_2O (≈10 equiv.)
did <u>not</u> give the unhydrolyzed N-acetyl-(ethyl)-glycinate

product by TLC (R_f=0.3, 100% EtOAc, yellow anisaldehyde stain).
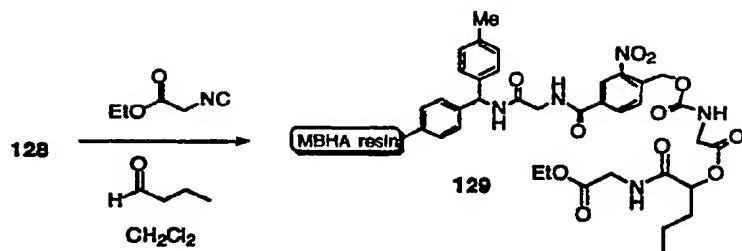
Methyl isocyanoacetate (23 μL, 0.23 mmol) and butyraldehyde (23 μL, 0.23 mmol) were added to a CH_2Cl_2

5 (200 μL) suspension of (glycinic acid)-Gly-MBHA resin **128** (25 mg, $\approx$0.025 mmol). The mixture was allowed to stand at 25°C for 48 hours. The resin was then filtered, washed exhaustively with sequential applications of $CH_2Cl_2/MeOH/CH_2Cl_2$ and dried *in vacuo*

10 overnight to afford (Mica-But-Gly)-Gly-MBHA resin **129** ($\approx$27 mg) as yellow beads.
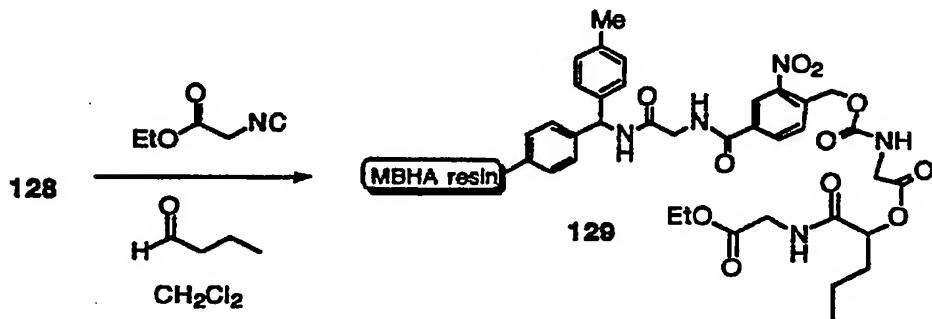
General procedure for the photolytic cleavage of *o*-nitrobenzyl carbamates and *in situ* acylation of Passerini adducts:

15   Acetic anhydride (10 equiv.) was added to a CH_3CN suspension of (Mica-But-Gly)-Gly-MBHA resin **128** (25

65

mg, ≈0.025 mmol) and irradiated at 350 nm in a Rayonet
for 12-48 hours at 30°C. The solution was transferred
into a round bottom flask by pipet with multiple
washings (CH₃CN) of the resin. The solvent, excess

5   acetic anhydride, and acetic acid was then efficiently
removed in vacuo to afford Mica-But-(N-acetyl-Gly)
adduct 129 (≈2-4 mg by crude ¹H NMR) as a colorless oil.
¹H NMR and TLC demonstrated this was the only compound
present.

10   129: ¹H NMR (360 MHz, CDCl₃), δ 6.93 (bt, J=5
Hz, 1H, NH), 6.11 (bt, J=5 Hz, 1H, NH), 5.28 (t, J=6.0
Hz, 1H, OCH), 4.13 (dd, J=5.7, 18.2 Hz, 1H, HNCH₂CO₂CH₃),
4.08 (5, J=5.5 Hz, 2H, HNCH₂CO₂CH₃), 3.97 (dd, J=5.3,
18.1 Hz, 1H, HNCH₂CO₂CH₃), 3.76 (s, 3H, CO₂CH₃), 2.07 (s,

15 3H, HNCOCH₃), 1.89 (m, 2H, OCHCH₂), 1.41 (m, 2H,
OCHCH₂CH₂), 0.93 (t, J=7.4 Hz, 3H, CH₂CH₃); LRMS (FAB)
[MH]⁺ calcd for C₁₂H₂₀N₂O₆ 289.14, found 289.5.

Example 12   Solid Phase Passerini MCCA Array
                  Synthesis Using The Photocleavable
20   Carbamate Linker Of Example 11
          A solid phase MCCA array synthesis using the
photocleavable carbamate linker of Example 11 was
conducted and is depicted in Table 4. In this
synthesis, 8 isocyanides, 6 aldehydes, 1 carboxylic acid

25 and 1 acylating agent were employed. It should be
understood that a variety of different carboxylic acids
and acylating agents could have been used.

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TLC and LRMS Data for Polymer Supported Passerini MCCAS

Core Structure

MBHA resin + R_1CHO + R_2-NC → (1. CH_2Cl_2 2. Irrad at 350 nm Ac_2O)

Isocyanide R_2-NC

Polymer Supported Carboxylic acid component in all wells

Isocyanide R_2-NC		A		B		C		D		E		F	
Ts—NC	8	+MN +T +MG		+MN +T		+MN +T		−MN −T		+MN +T		−MN +T	
PhCH₂—NC	7	+MN +T +MG		+MN +T		−MN +T		−MN −T		+MN −T −MG		+MN −T	
⟍⟍—NC	6	−MN +T −MG		+MN +T +MG		−MN +T −MG		+MN −T		−MN −T −MG		−MN −T −MG	
⟍⟍⟍—NC	5	+MN +T		+MN +T		−MN +T +MG		−MN −T		−MN −T +MG		−MN −T	
MeO...Ph N Me —NC	4	−MN −T −MG		−MN −T		−MN −T		−MN −T		−MN −T −MG		−MN −T	
MeO...Ph N —NC	3	−MN −T −MG		−MN −T		+MN −T −MG ⊖		−MN −T		−MN −T		−MN −T	
MeO...Ph Br —NC	2	+MN +T +MG		+MN +T ⊕		+MN +T		−MN −T		+MN +T −MG		−MN −T	
MeO...—NC	1	+MN +T +MG ⊕		+MN +T ⊕		+MN +T ⊕		−MN −T		+MN +T		+MN +T	

aldehyde R_1CHO ⇨

+T = TLC showed expected spot(s); −T = TLC did not show expected spot(s);
+MN= LR Mass Spectra (NBA matrix) showed expected product; −MN= LR
Mass Spectra (NBA matrix) did not showed expected product; +MG= LR Mass
Spectra (Glycerol matrix) showed expected product; − MG= LR Mass Spectra
(Glycerol matrix) did not showed expected product; ⊕='H NMR showed
expected product; ⊖ ='H NMR did not showed expected product.

All reactions were performed in Fisher shell vials arranged in two 4x6 microtiter trays set side by side to form the requisite 8x6 array. 25mg of resin was added to each vial and swelled with CH_2Cl_2. The
5 aldehydes and carboxylic acids were then delivered by gas tight syringe to each of their respective rows and columns and allowed to react at room temperature for 2-4 days. Filtration of the individual reactions and exhaustive washings followed by photolysis at 350 nm in
10 CH_3CN (10 equiv. Ac_2O) for 24h gave 48 reaction wells to be analyzed for product composition. The results of the array synthesis is depicted in Table 4. Low resolution mass spectroscopy combined with thin layer chromatography of each of the reaction wells showed that
15 50% of the desired products were formed and isolated. ^{1}H NMR of the reaction wells that gave positive TLC and mass spectroscopy data revealed that the desired products were the only solid phase products produced in these wells. This example thus demonstrates that large
20 arrays of compounds can be rapidly and efficiently prepared in high purity using solid phase synthesis according to the present invention.

While the invention of this patent application is disclosed by reference to specific MCCA reactions, it
25 is understood that the present invention can be applied all other multicomponent syntheses capable of being conducted in a single reaction vessel without the isolation of an intermediate product. Further, the present invention is intended to be applicable to all
30 future developed MCCA reactions.

It is also understood that the embodiment of the present invention encompassing solid phase synthesis is intended to be practiced with the linkers and solid supports disclosed herein as well as with other linkers
35 and solid supports presently known or developed in the future.

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Accordingly, the invention may be embodied in other specific forms without departing from its spirit or essential characteristics. It is to be understood that this disclosure is intended in an illustrative
5 rather than limiting sense, as it is contemplated that modifications will readily occur to those skilled in the art, within the spirit of the invention and the scope of the appended claims.

.69

What is claimed is:

       1.   An array of compounds having a common core structure wherein the compounds of the array comprise the products of a multiple component
5   combinatorial array synthesis having at least three components wherein the components of the multiple component combinatorial array synthesis comprise

       a first component, the first component comprising a first group of reactants having a same
10 first functional group;

       a second component, the second component comprising a second group of reactants having a same second functional group;

       a third component, the third component
15 comprising a third group of reactants, the third group of reactants having a same third functional group;

wherein the array synthesis is conducted under appropriate conditions such that the functional groups of the first, second and third components react with
20 each other to form the array of compounds having a common core structure.

       2.   The combinatorial array of compounds of claim 1 wherein the first group of reactants comprises more than one reactant and the second group of reactants
25 comprises more than one reactant.

       3.   The combinatorial array of compounds of claim 1 wherein the reactants of the first component comprise the compounds of another combinatorial array of compounds.

30   4.   The combinatorial array of compounds of claim 1 wherein the reactants of at least one component comprise mixtures of more than one compound having the same functional group.

5.   The combinatorial array of compounds of claim 1 wherein each compound is formed while bound to a solid support.

6.   The combinatorial array of compounds of claim 5 wherein the first group of reactants comprises more than one reactant and the second group of reactants comprises more than one reactant.

7.   A method of making a combinatorial array of compounds having a common core structure using an n component combinatorial array synthesis, wherein n corresponds to the number of reaction components, n being at least 3, wherein each component comprises a group of reactants having the same functional group; the method comprising:

a) organizing a series of reaction vessels in an n dimensional array wherein each reaction vessel is identifiable by its coordinates in the n dimensional array, each axis in the n dimensional array corresponding to a different component, each position on the axis corresponding to a different reactant;

b) adding the reactants of the n components to the n dimensional array of reaction vessels such that the same reactant is added to all of the reaction vessels in the array having a position on the array corresponding to that reactant;

c) reacting the contents of each reaction vessel under appropriate conditions to form the compounds of the array.

8.   The method of claim 7 comprising the further step of binding one of the components to a solid support.

9.   The method of claim 7 wherein the reactants of at least one component comprise mixtures of more than one compound having the same functional group.

10.   The method of claim 7 wherein the reactants of the first component comprise the compounds of another combinatorial array of compounds.

11.   The method of claim 7 wherein at least two of the components comprise a group of more than one reactant.

12.   The method of claim 11 comprising the further step of binding one of the components to a solid support within each reaction vessel of the array.

13.   A method of creating a combinatorial array of compounds with a common core structure comprising:
          a.   identifying the desired core structure;
          b.   identifying a MCCA reaction capable of generating the core structure;
          c.   preparing an array of compounds using the identified MCCA reaction according to the method of claim 7.

14.   A method of creating a combinatorial array of compounds with a common core structure comprising:
          a.   identifying the desired core structure;
          b.   identifying a MCCA reaction capable of generating the core structure;
          c.   preparing a combinatorial array of compounds using the identified MCCA reaction according to the method of claim 8.

15. A method of creating a combinatorial array of compounds with a common core structure comprising:

a. identifying the desired core structure;

b. identifying a MCCA reaction capable of generating the core structure;

c. preparing a combinatorial array of compounds using the identified MCCA reaction according to the method of claim 9.

16. A method for conducting <u>in vitro</u> binding studies on a biological material comprising:

a) adding the biological material to an array of compounds, each compound in the array having a common core structure and being bound to a solid support; and

b) measuring the binding of each compound in the array to the biological material.

17. A linker bound to a polymer useful for binding compounds during solid phase synthesis, the linker having the chemical structure

$$[POLYMER] - HNCOCH_2CH_2CH(CH_2CH_2N_3)CO_2H$$
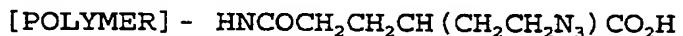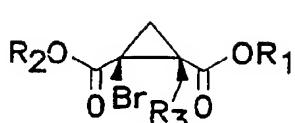
FIG. 1A
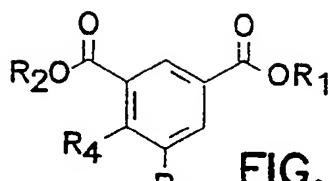
FIG. 1B
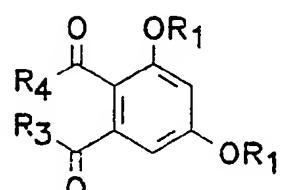
FIG. 1C
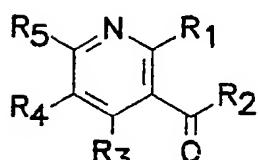
FIG. 1D
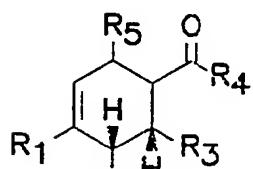
FIG. 1E
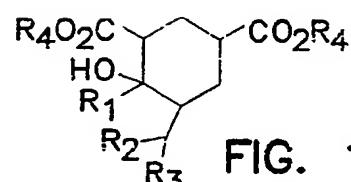
FIG. 1F
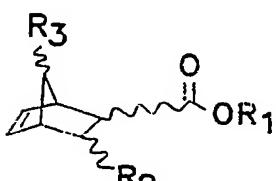
FIG. 1G
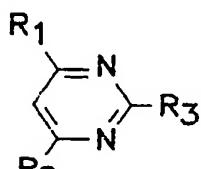
FIG. 1H
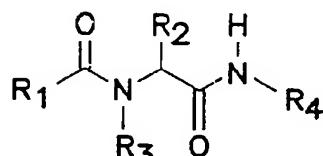
FIG. 1I
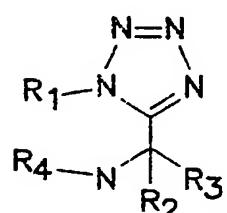
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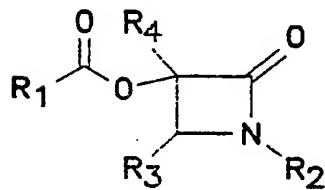
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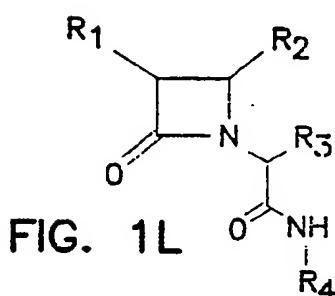
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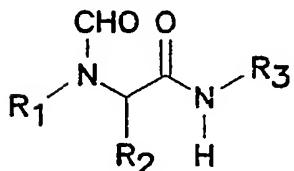
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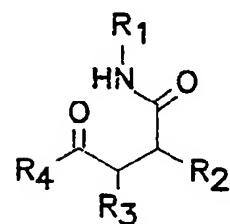
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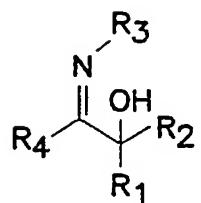
FIG. 1O
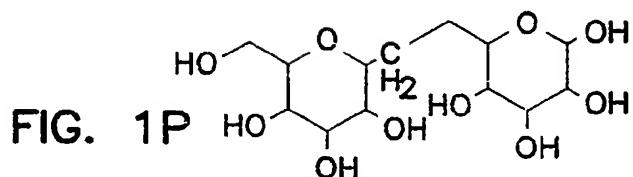
FIG. 1P
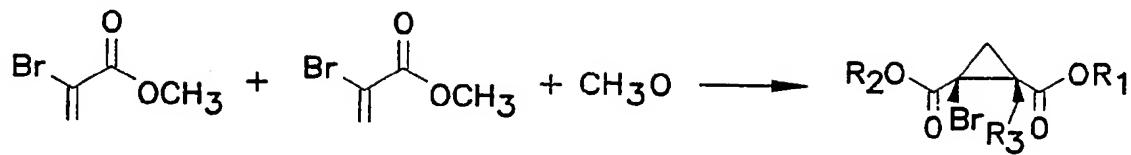
FIG. 2A
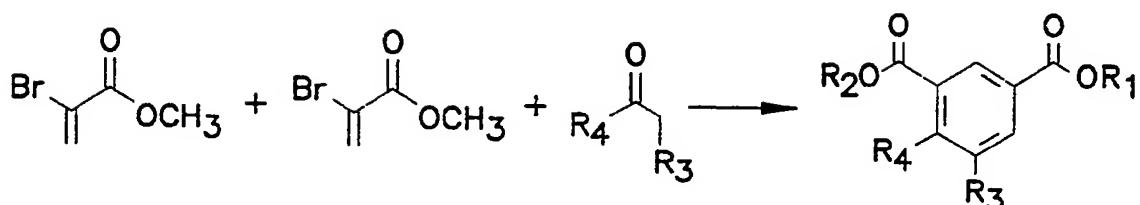
FIG. 2B
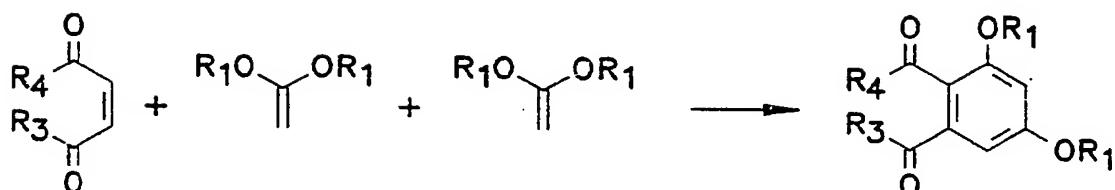
FIG. 2C
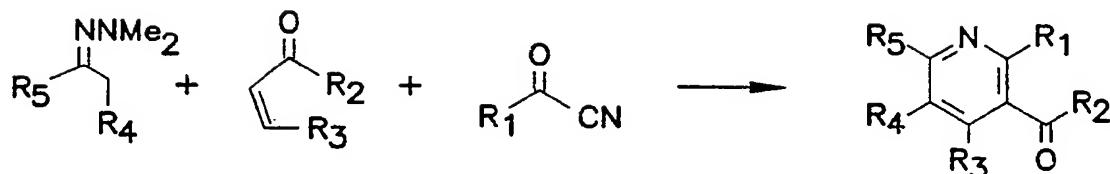
FIG. 2D
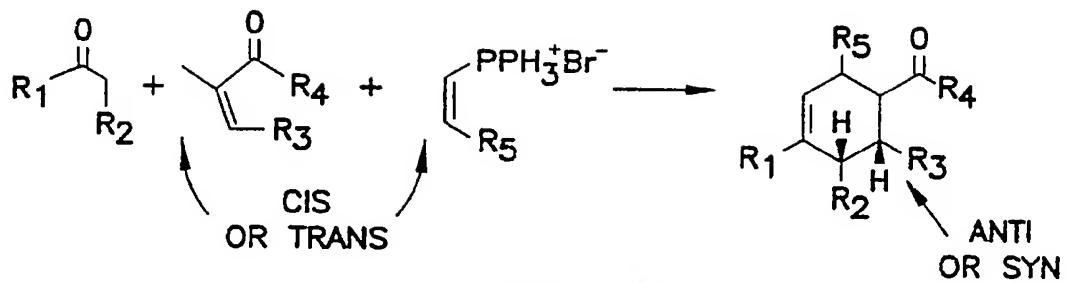
FIG. 2E
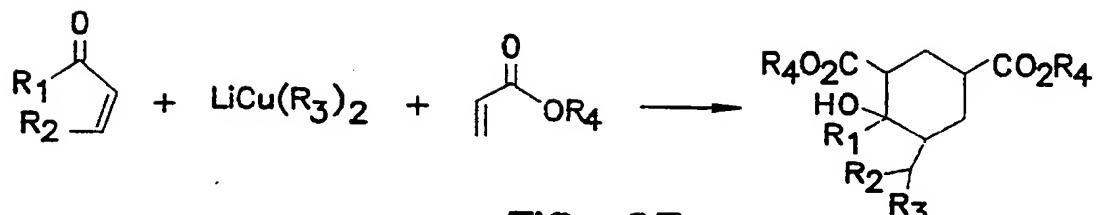
FIG. 2F
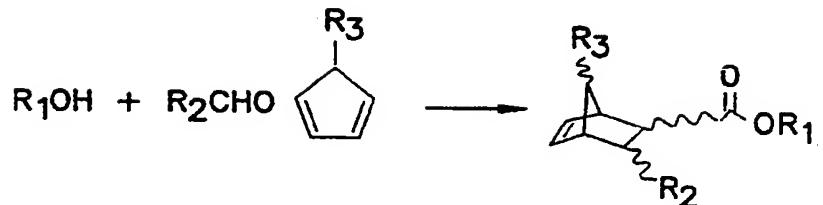
FIG. 2G
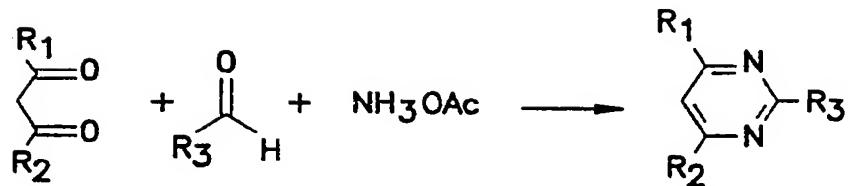
FIG. 2H

FIG. 2I

FIG. 2J

FIG. 2K

FIG. 2L

$$R_1NH_2 + R_2CHO + HCO_2H + R_2NO \longrightarrow$$

FIG. 2M

$$R_4CO_2H + R_1NC + R_2CHO + R_3NH_2 \longrightarrow$$

FIG. 2N

$$R_1\text{C(=O)}R_2 + R_3NH_2 + R_4X \longrightarrow$$

FIG. 2O

+ OXIDATION CATALYST

FIG. 2P

$$\begin{bmatrix} A^1 \\ A^2 \\ A^3 \end{bmatrix} \times \begin{bmatrix} B^1 \\ B^2 \\ B^3 \end{bmatrix} \times \begin{bmatrix} C^1 \\ C^2 \\ C^3 \end{bmatrix} \implies$$

$$3 \qquad 3 \qquad 3 = 27 \text{ ANALOGUES}$$

$A^1B^1C^3$	$A^1B^2C^3$	$A^1B^3C^3$
$A^2B^1C^3$	$A^2B^2C^3$	$A^2B^3C^3$
$A^3B^1C^3$	$A^3B^2C^3$	$A^3B^3C^3$

$A^1B^1C^2$	$A^1B^2C^2$	$A^1B^3C^2$
$A^2B^1C^2$	$A^2B^2C^2$	$A^2B^3C^2$
$A^3B^1C^2$	$A^3B^2C^2$	$A^3B^3C^2$

$A^1B^1C^1$	$A^1B^2C^1$	$A^1B^3C^1$
$A^2B^1C^1$	$A^2B^2C^1$	$A^2B^3C^1$
$A^3B^1C^1$	$A^3B^2C^1$	$A^3B^3C^1$
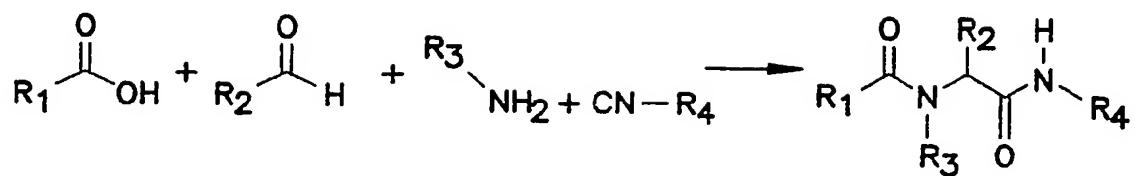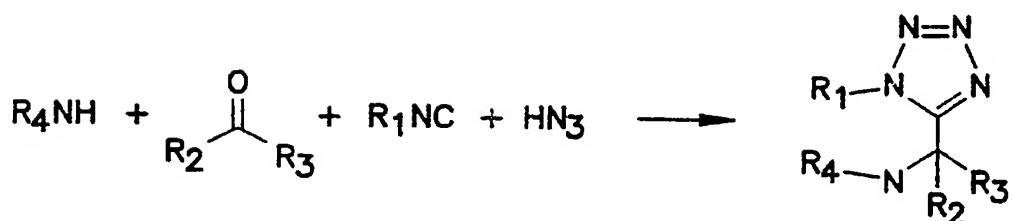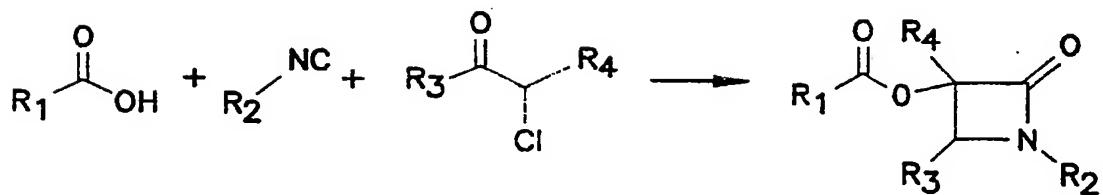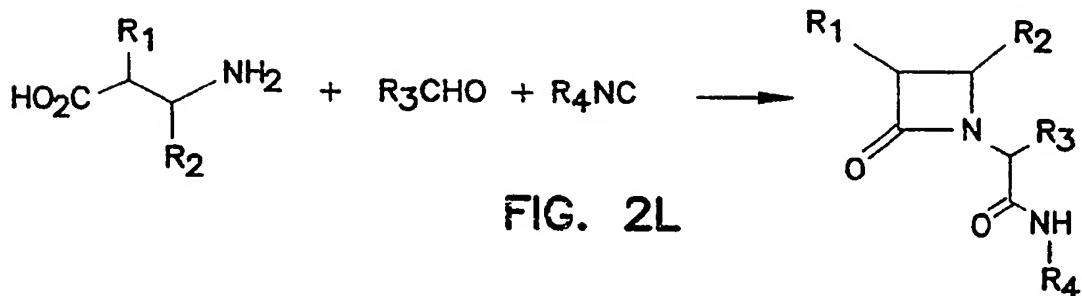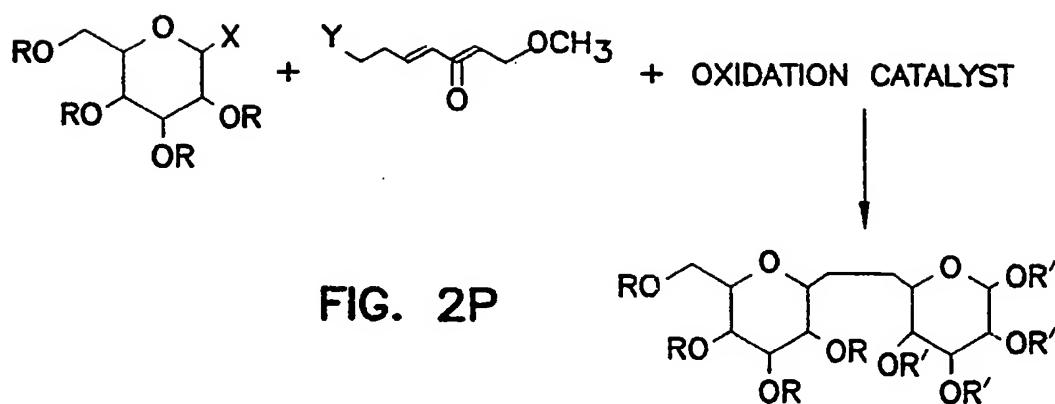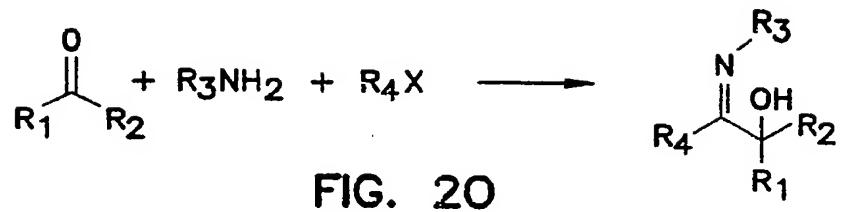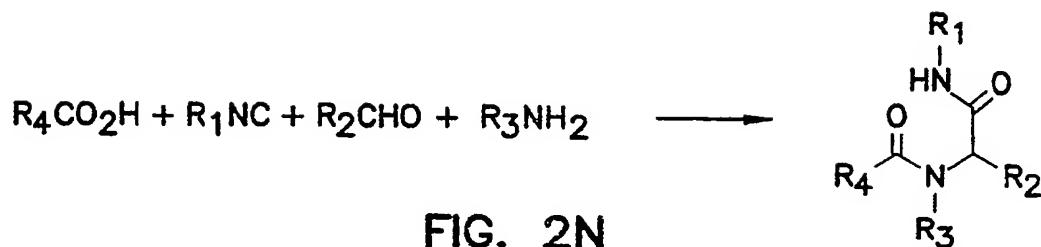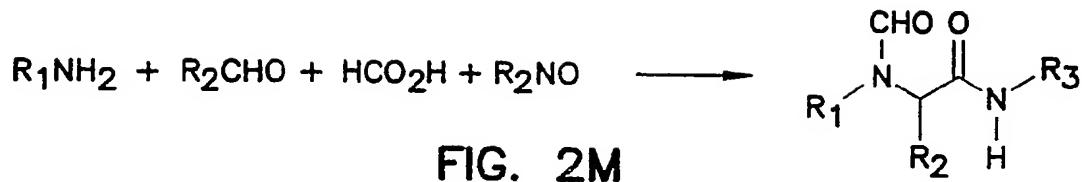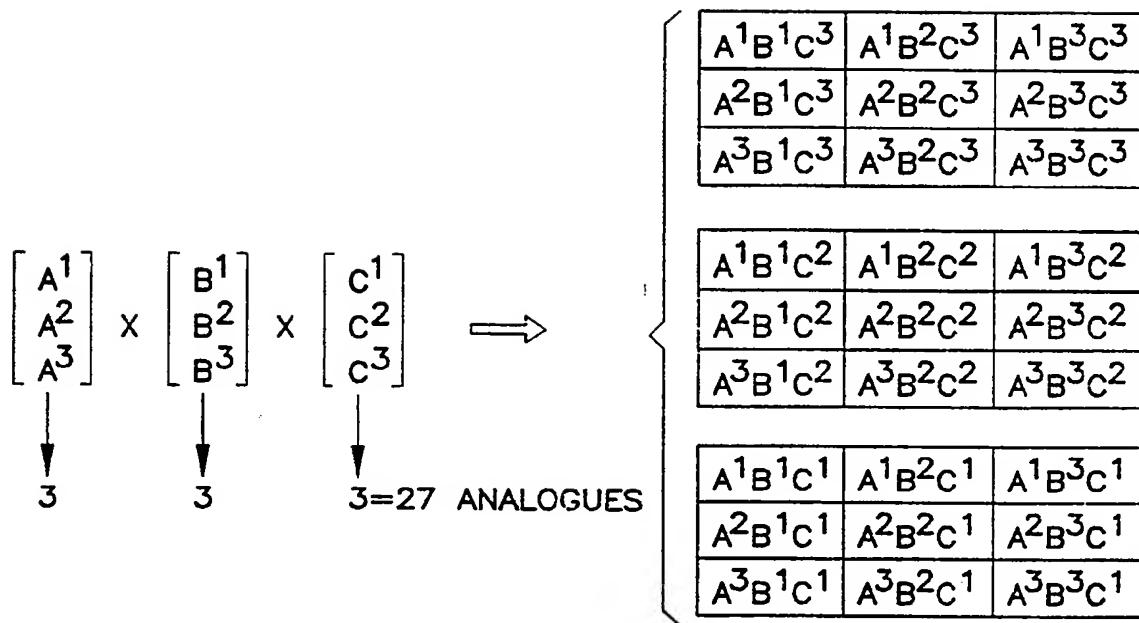
NUMBER OF REAGENTS PER COMPONENT A, B, C	NUMBER OF ANALOGUES PRODUCED
3, 3, 3	27
5, 5, 5	125
10, 10, 10	1000
20, 20, 20	8000
x, y, z	x*y*z
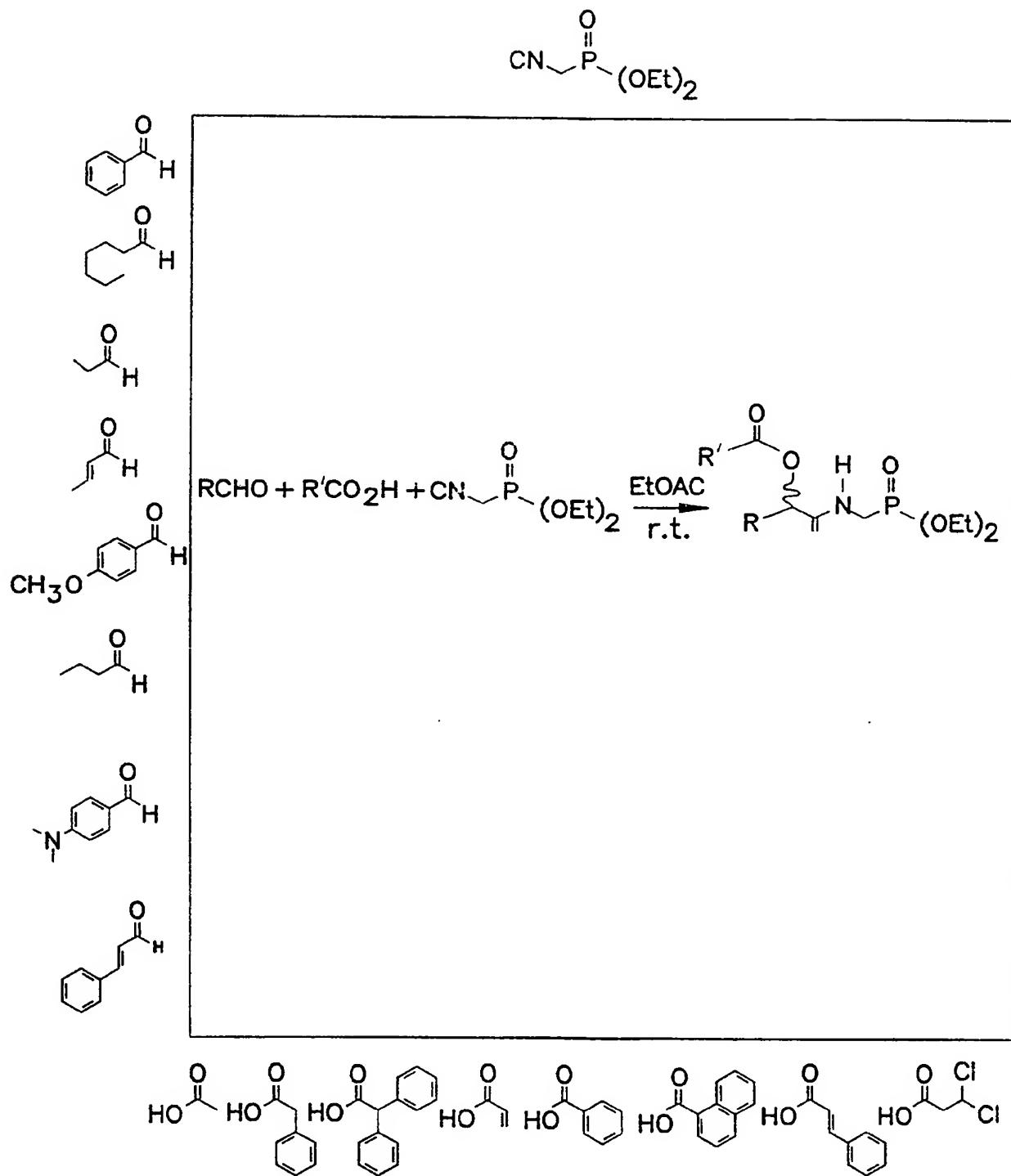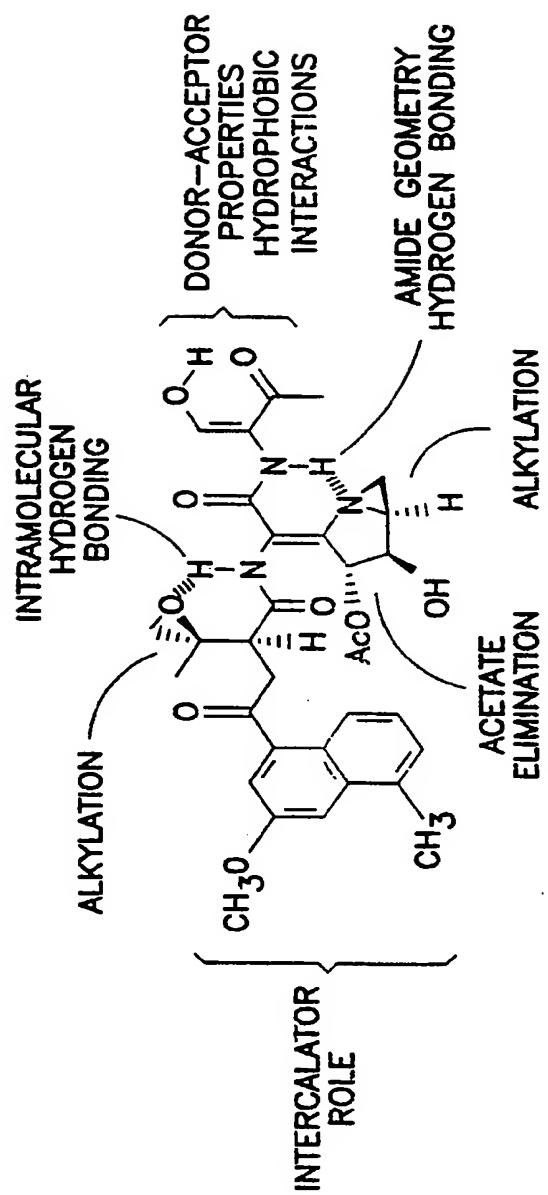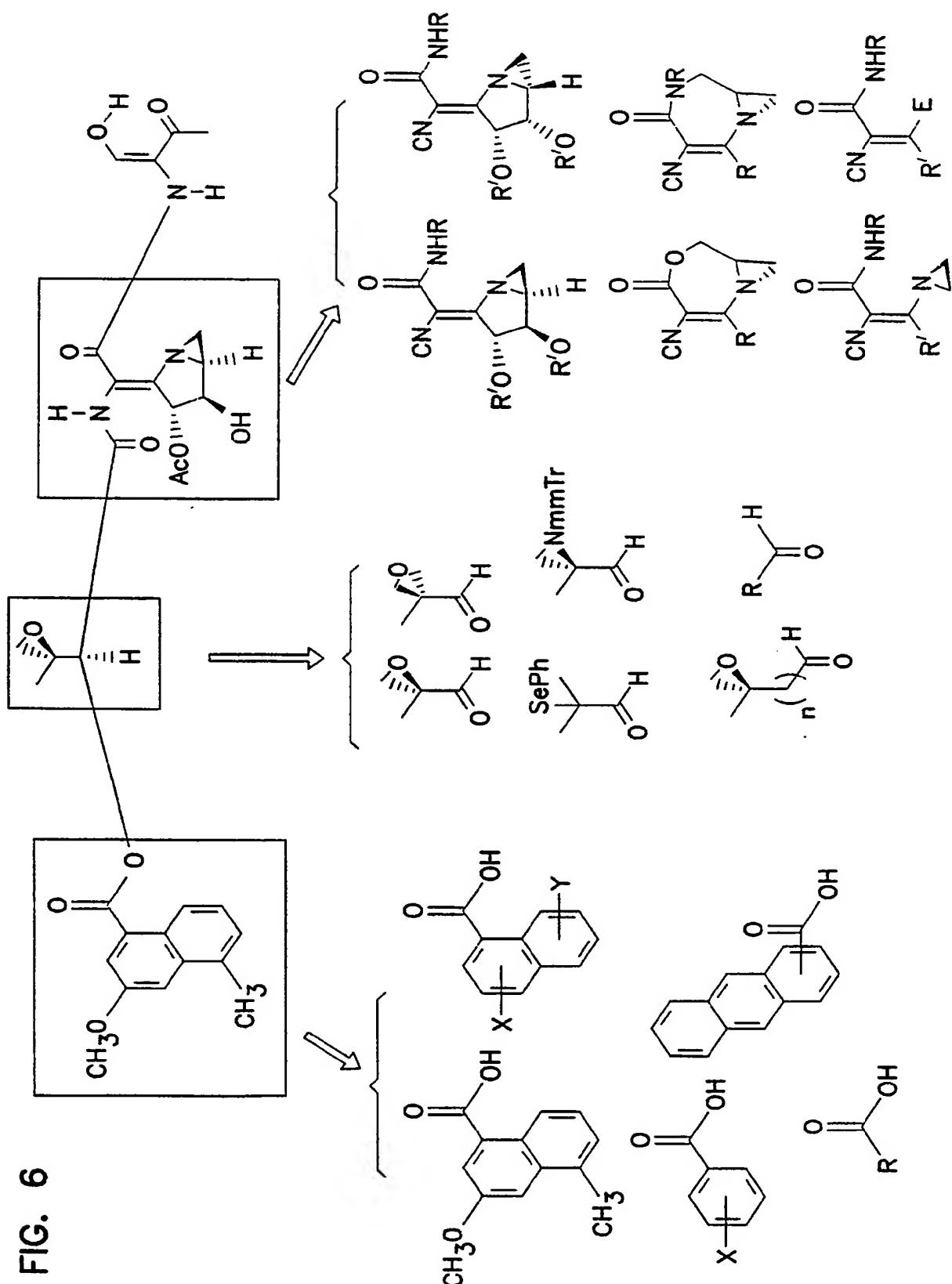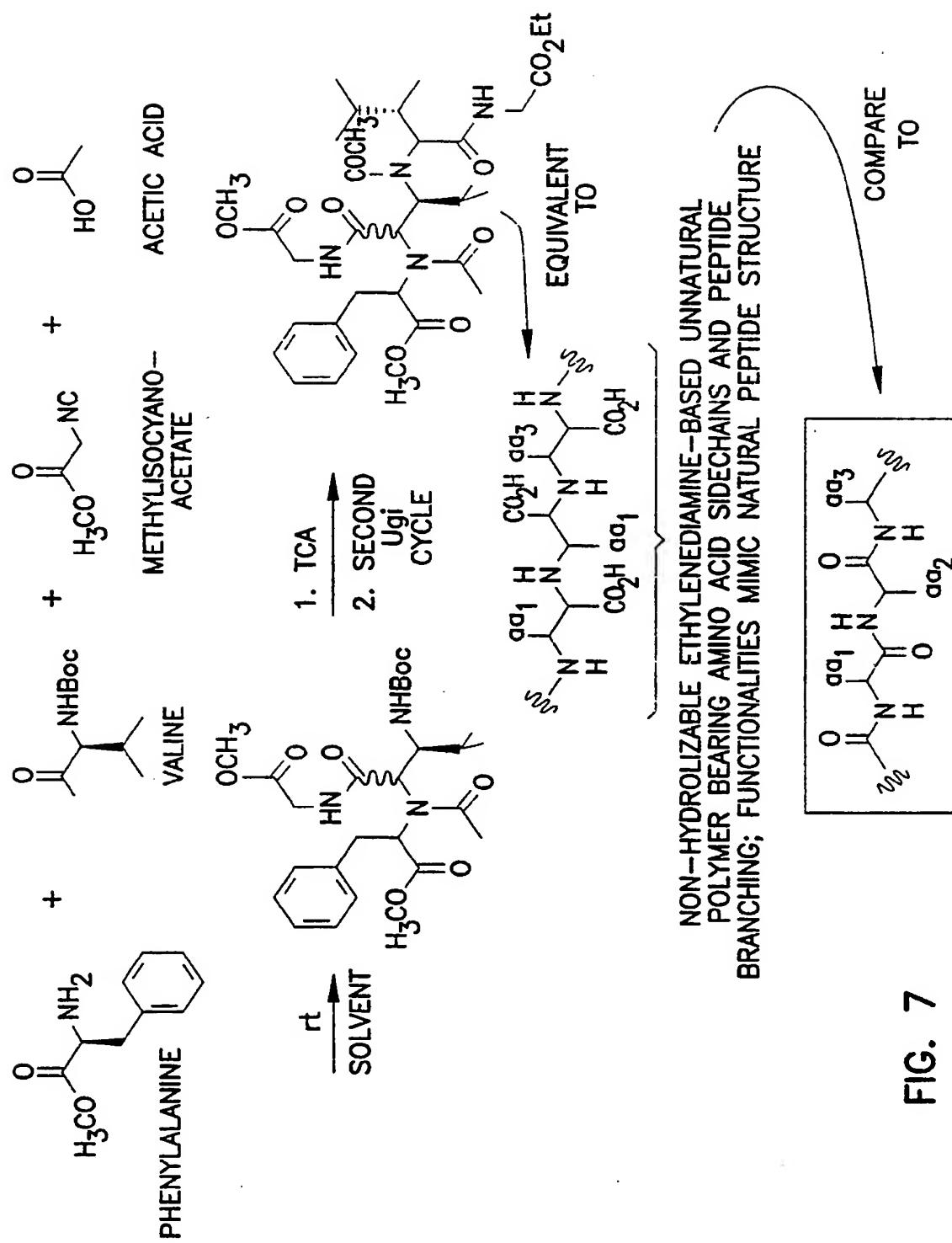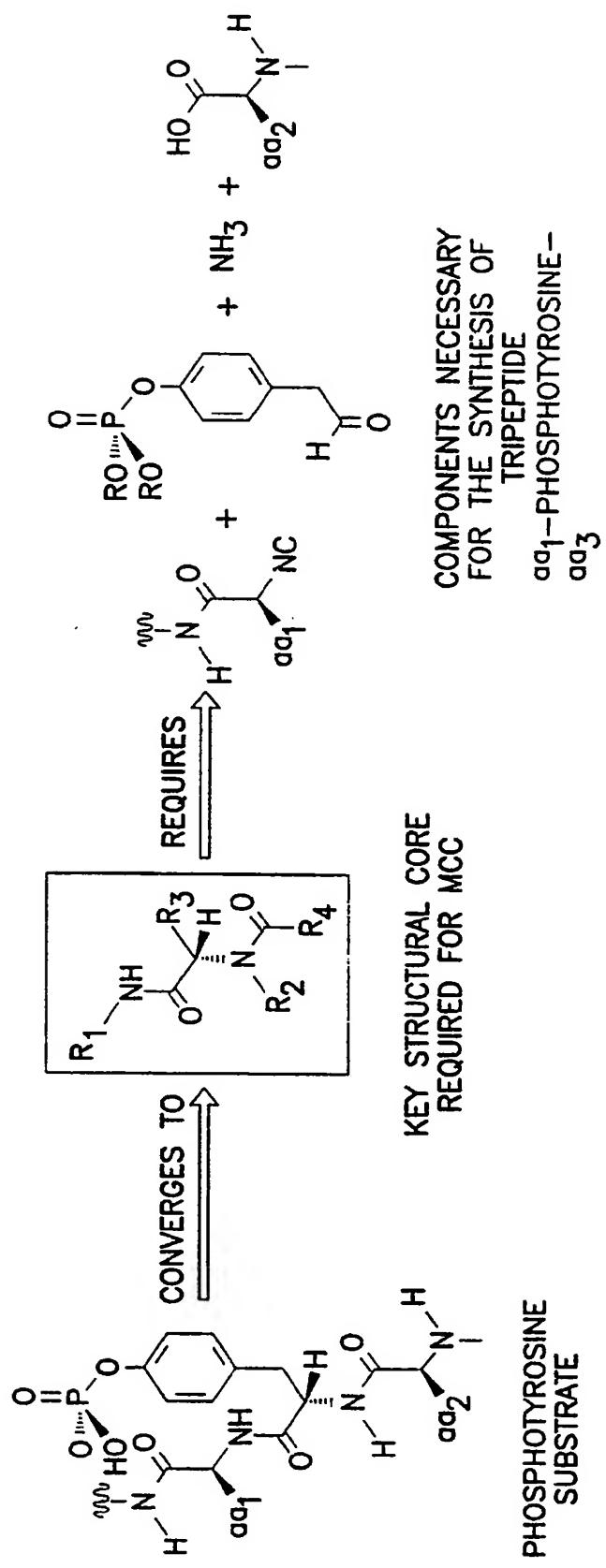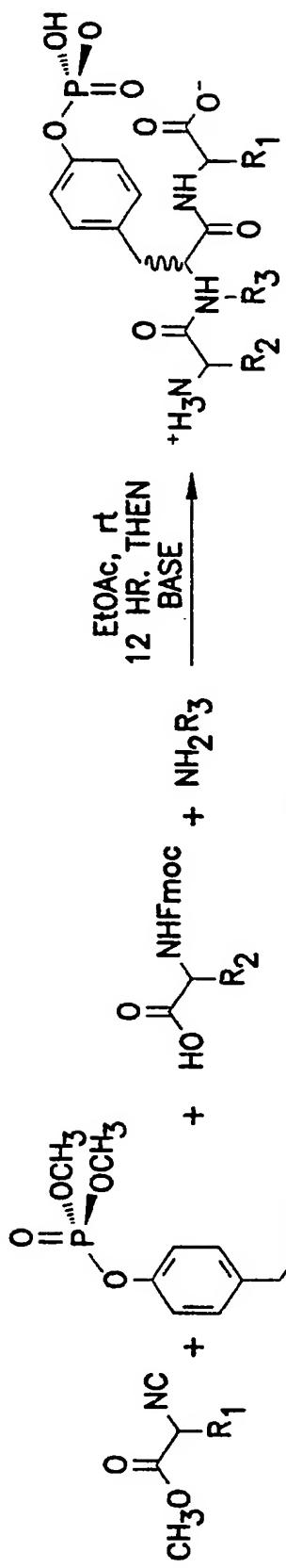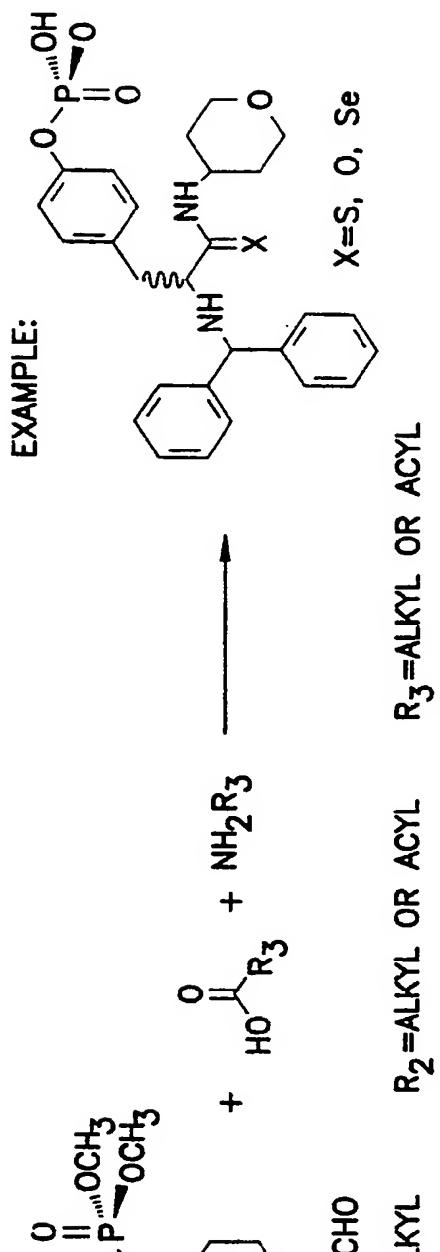
FIG. 3

FIG. 4

FIG. 5

FIG. 6

FIG. 7

FIG. 8

FIG. 9A

$$EtOAc, rt \\ 12 \ HR. \ THEN \\ BASE$$

$$+ \ NH_2R_3$$

FIG. 9B

EXAMPLE:

X=S, O, Se

$$+ \ NH_2R_3$$

R_2=ALKYL OR ACYL R_3=ALKYL OR ACYL

R_1=ALKYL

FIG. 9C

FIG. 9D

FIG. 10A

1. LITHIUM DIISOPROPYL AMIDE
TETRAHYDROFURAN, −78°C
N₂

2. ETHYLENE OXIDE

FIG. 10B

1. METHANESULFONYL
CHLORIDE, CH₂CL₂
TIETHYLAMINE

2. SODIUM AZIDE

AMINOMETHYL
DIVINYLSTYRENE
RESIN, 200 MESH

DMF, HEAT

POLYMER

FIG. 10C

DICYLOHEXYLCARBODIIMIDE
HYDROXYBENZYLTRIAZOLE
ALCOHOL, AMINE, MERCAPTAN

POLYMER

X=S,N,O,Se
R=ALKYL, ALLYL, ARYL

POLYMER

1. DICYCLOHEXYLCARBODIIMIDE DMF, HYDROXYBENZYLTRIAZOLE
2. SODIUM HYDROXIDE

TRIPHENYLPHOSPHINE OR HYDROGENATION H_2/Pd, P

HEAT (OR ROOM TEMPERATURE)

FIG. 10C CONTINUED
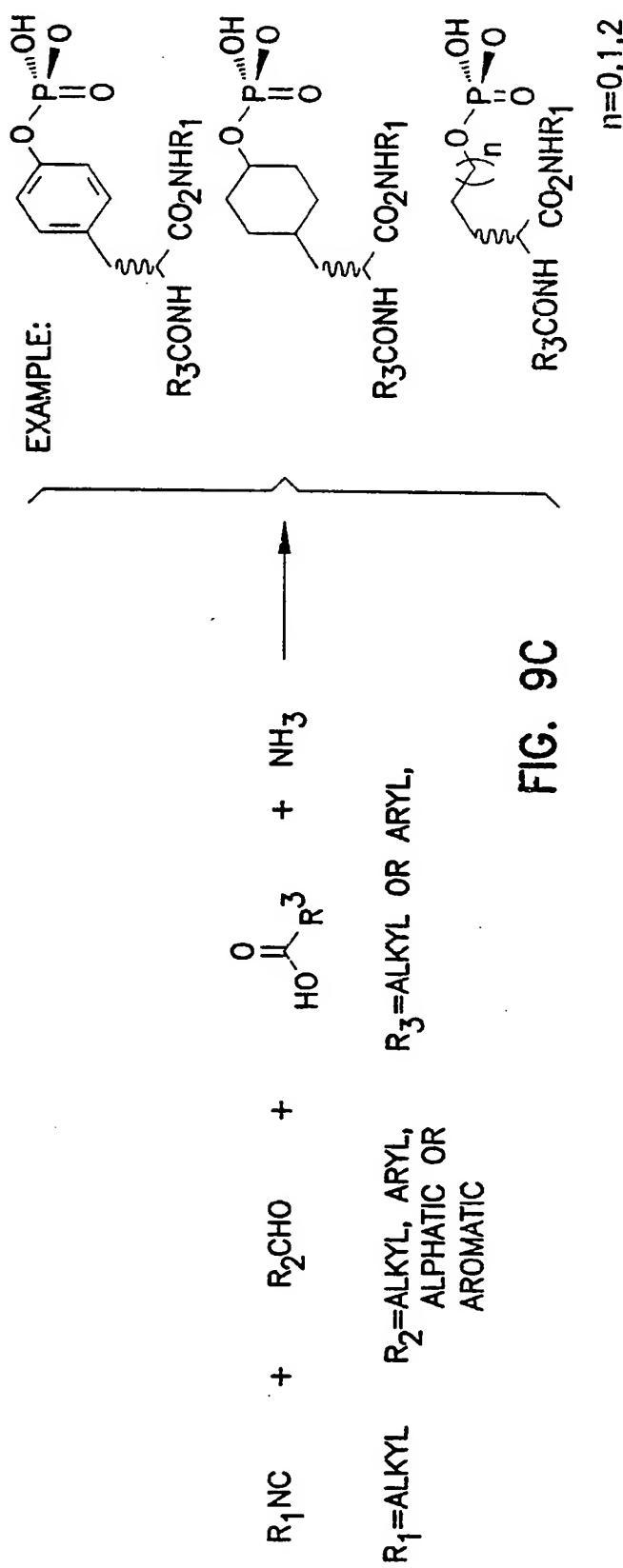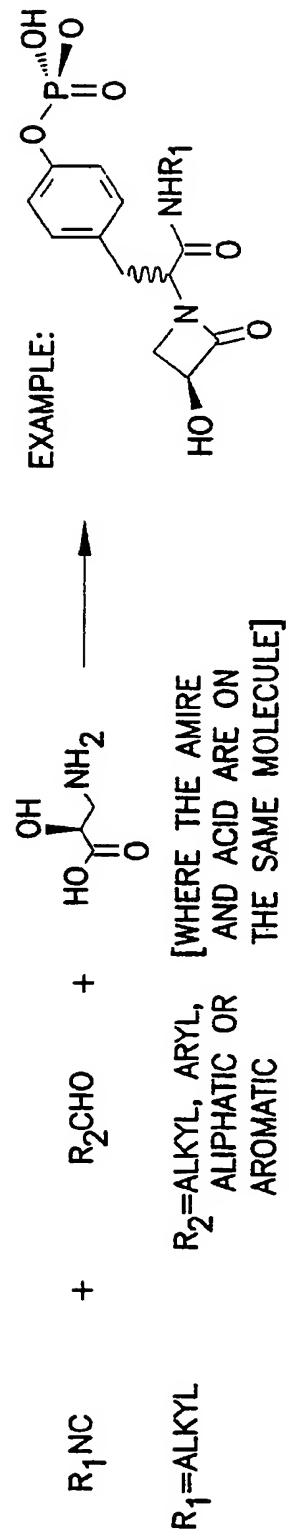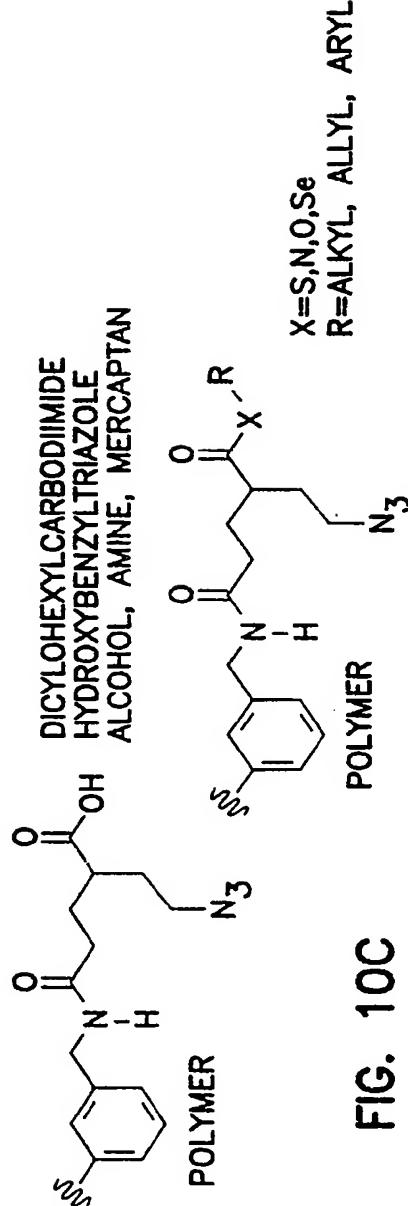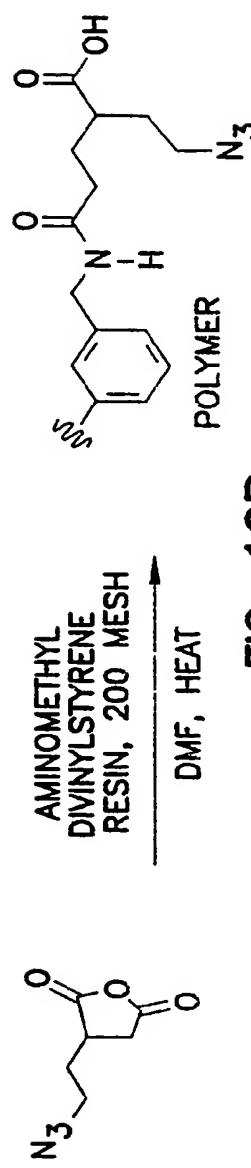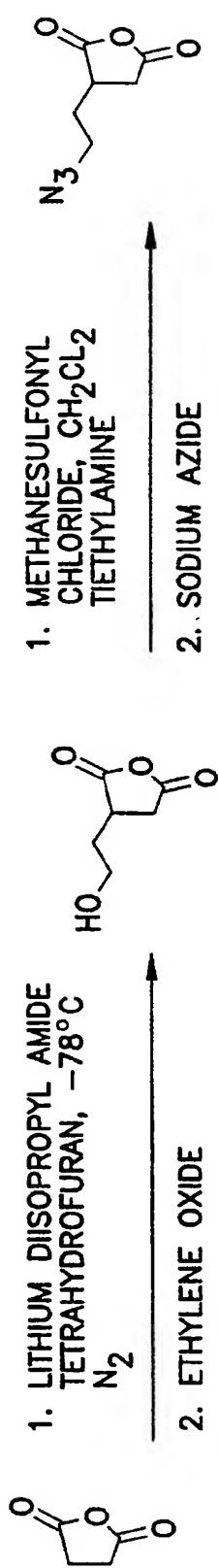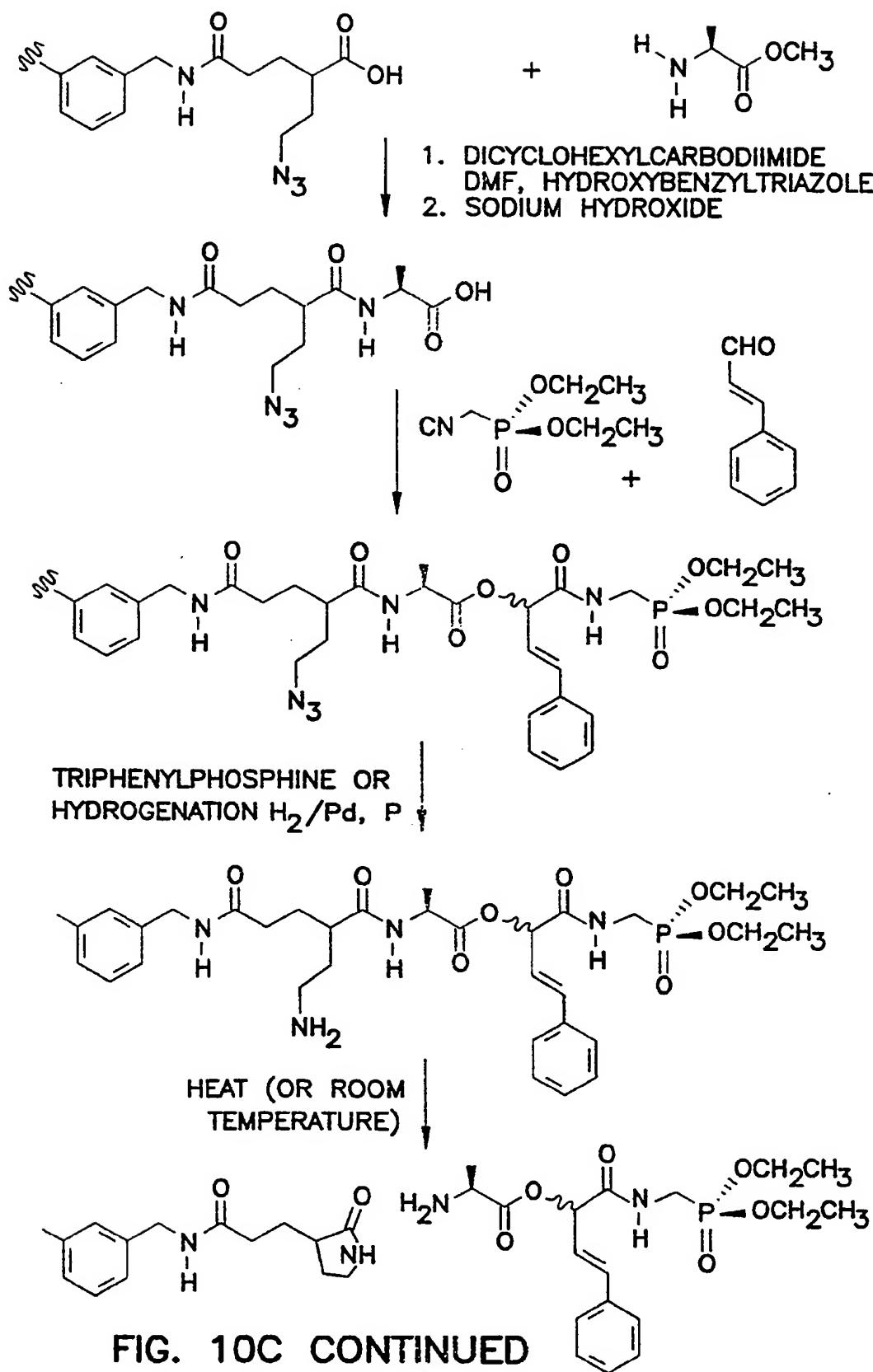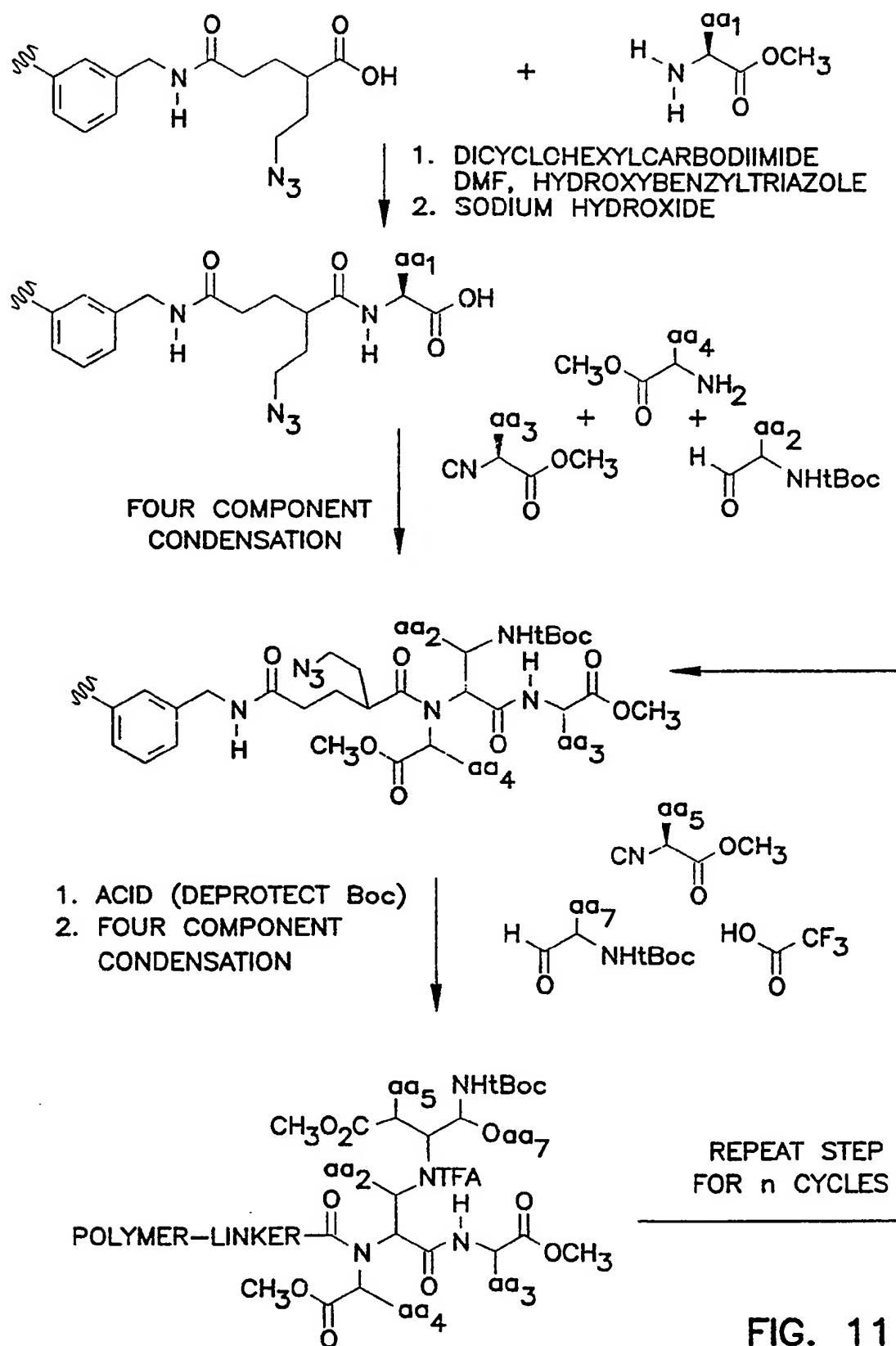
FIG. 11

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   C07B43/06   B01J19/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   C07B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category°	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol.114, 1992, WASHINGTON, DC US pages 10997 - 10998 J. A. ELLMAN, B. A. BUNIN 'A General and expedient method for the solid-phase synthesis of 1,4-Benzodiazepine derivatives' see the whole document ---	1,2,4-6, 13-15
A	PEPTIDE RESEARCH, vol.6, no.3, June 1993 pages 161 - 170 V. NIKOLAIEV ET AL. 'Peptide encoding for structure determination of nonsequenceable polymers within libraries synthesized and tested on solid-phase supports' --- -/--	1-17

[X] Further documents are listed in the continuation of box C.   [ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 November 1994	2 5. 11. 94

| Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016 | Authorized officer Seufert, G |

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NATURE, vol.354, 7 November 1991, LONDON GB pages 84 - 86 R. A. HOUGHTON ET AL. 'Generation and use of synthetic peptide combinatorial libraries for basic research and drug discovery' see the whole document	7,16
A	NATURE, vol.354, 7 November 1991, LONDON GB pages 82 - 83 K. S. LAM ET AL. 'A new type of synthetic peptide library for identifying ligand-binding activity' see the whole document	7,16
A	ANGEWANDTE CHEMIE. INTERNATIONAL EDITION, vol.31, no.4, 1992, WEINHEIM DE pages 367 - 486 G. JUNG, A. G. BECK-SICKINGER 'Multiple peptide synthesis methods and their applications'	7,16
P,X	PROC. NATL. ACAD. SCI., vol.90, August 1993 pages 6909 - 6913 S. HOBBS DEWITT ET AL. 'Diversomers: an approach to nonpeptide, nonoligomeric chemical diversity'	1-16
T	JOURNAL OF MEDICINAL CHEMISTRY, vol.37, no.10, 13 May 1994, WASHINGTON US pages 1385 - 1401 E. M. GORDEN ET AL. 'Application of combinatorial technologies to drug discovery. 2. Combinatorial organic synthesis, library screening strategies, and future directions' see the whole document, especially page1390 - pqge 1392	

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